UNIVERSITY OF PENNSYLVANIA

STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

HOWARD TINT

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STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. I. THE COMPARATIVE VIRULENCE OF CERTAIN FUSARIA[1, 2]

HOWARD TINT[3]

(Accepted for publication January 15, 1945)

INTRODUCTION

In the many reports of the relationship between Fusaria and damping-off in conifers, either observed or determined by inoculation experiments, the repeated occurrence of certain species suspected of being causal agents has led to the general acceptance of the pathogenic nature of a few of these. According to a compilation by Wollenweber and Reinking (32), of importance in Europe are *Fusarium oxysporum* v. *aurantiacum,* synonymous with *Fusoma parasiticum* Tubeuf (4, 29, 30, 31) and *Fusoma pini* Hartig (2, 9, 10), *F. oxysporum* v. *aurantiacum* f. 1 (*F. macroxysporum* Lindfors) (16), *F. bulbigenum* v. *blasticola* (*F. blasticola* Rostrup) (24), *F. culmorum, F. avenaceum* (*F. venenorum*) (6), *F. sambucinum, F. scirpi* v. *acuminatum, F. fuligenosum* and *F. echinosporum* (25), and *F. graminearum* (*Giberella saubinetii*) (19); and in the United States primarily *F. sporotrichioides, F. arthrosporioides, F. sambucinum* f. 6 (*F. discolor sulphureum* Schl.), and to a lesser extent *F. culmorum, F. solani, F. orthoceros, F. moniliforme, F. oxysporum, F. vasinfectum* and *F. graminearum* (12, 13, 20, 21, 26). These constitute only a small selection of the many species mentioned in the literature, and there is need for further tests with some of them as well as with other Fusaria, in order to determine which species are really parasitic and how they compare with other damping-off fungi used as standards. The object of the present investigation has been to carry out such tests by various critically studied experimental methods. Where more than one host was inoculated, relative susceptibility to infection was tested.

The effect upon the experimental design of limitations in time and space made it infeasible to consider damping-off losses according to the classical symptomatic categories (12, 13, 28): germination loss, normal or ordinary damping-off, root-rot or late damping-off, and top damping-off. Experiments were maintained long enough to test ability to induce normal damping-off, but probably not long enough to test potentialities of the Fusaria as root-rotting organisms. Damping-off losses were in two classes: (I) emergence loss, the reduction in the number of seedlings appearing above the surface of the substrate as compared with the total number of viable seeds, the latter determined by the emergence in uninoculated controls. This

[1] A portion of a dissertation presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to express appreciation to: Dr. H. H. York under whose direction the investigation was carried out; Dr. W. G. Hutchinson for criticism of the manuscript; Dr. P. V. Lufkin for assistance on statistical analysis; and Dr. C. D. Sherbakoff for identifications of some Fusaria used in the investigations.

[3] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

TABLE 1.—*Identity and sources of fungi tested for ability to cause damping-off of coniferous seedlings*

Species[a]	Line	Host source	Year of isolation	Locality	Isolator[b]
Fusarium aquaeductuum (Radlk. & Rabh.) Lagh. v. *medium* Wr.	1C[c]	Red pine (discolored wood of stem, 16-yr.-old tree)	1932	Hemlock Lake, N. Y.	Y
F. arthrosporioides Sherb.	52	Scotch pine (damping-off)	1940	Washington Crossing, N. J.	T
F. avenaceum (Fr.) Sacc.	51[c]	" " " "	"	" " " "	T
	59[c]	" " " "	"	" " " "	T
	64[c]	" " " "	"	" " " "	T
	66	" " " "	"	" " " "	T
	69[c]	" " " "	"	" " " "	T
F. equiseti (Cda.) Sacc.	27	White pine (damping-off)	"	Philadelphia, Pa.	K
F. graminearum Schwabe	50	Scotch pine (damping-off)	"	Washington Crossing, N. J.	T
F. javanicum Koord. v. *radicicola* Wr.	R32	American Type Culture Coll.	.....	.....	.....
F. moniliforme Sheld.	R35	" " " "	.....	.....	.....
F. orthoceros App. & Wr.	16[c]	White pine (discolored wood of root)	1937	Hemlock Lake, N. Y.	Y
	R23A[c]	Red pine (root rot, 1–2-yr.-old seedlings)	1939	Saratoga Springs, N. Y.	E
	28[c]	White pine (damping-off)	1940	Philadelphia, Pa.	K
F. orthoceros App. & Wr. v. *longius* (Sherb.) Wr.	R60	Scotch pine (damping-off)	"	Washington Crossing, N. J.	T
	61	" " " "	"	" " " "	T
F. oxysporum Schl.	30	American Type Culture Coll.	.....	.....	.....
F. poae (Peck) Wr.	R11C[c]	Red pine (extra-seasonal growth (15), in 1935 node)	1936	Hemlock Lake, N. Y.	Y
	68[c]	Scotch pine (damping-off)	1940	Washington Crossing, N. J.	T

TABLE 1.—(*Continued*)

Species[a]	Line	Host source	Year of isolation	Locality	Isolator[b]
F. reticulatum Mont.	26A	White pine (damping-off)	1940	Philadelphia, Pa.	K
	58[c]	Scotch pine (damping-off)	"	Washington Crossing, N. J.	T
	62	" " " "	"	" " " "	T
F. sambucinum Fuckel	R8A[c]	Norway spruce (wood 1-in. root of 26-yr.-old tree)	1937	Hemlock Lake, N. Y.	Y
F. scirpi Lamb. & Fautr. v. *acuminatum* (Ell. & Ev.) Wr.	R22[c]	Red pine (stem wood of 1925 node)	1931	" " " "	Y
F. solani (Mart.) App. & Wr.	R9B[c]	White pine (inner bark of root crown)	1932	Norwich, N. Y.	Y
F. solani (Mart.) v. *Martii* (App. & Wr.) f. 1 Wr.	R2A[c]	White pine (wood of $\frac{1}{8}$-in. root of 22-yr.-old tree)	1936	" " "	Y
F. solani (Mart.) App. & Wr. v. *minus* Wr.	67	Scotch pine (damping-off)	1940	Washington Crossing, N. J.	T
F. sporotrichioides Sherb.	R33	American Type Culture Coll.	.....	.....	.....
F. vasinfectum Atk.	R34	" " " "	.....	.....	.....
Penicillium sp.	CI	Greenhouse soil	1940	Philadelphia, Pa.	T
Pythium ultimum Trow.	1145	Douglas fir (damping-off)	1925	Monument, Colorado	J
Rhizoctonia solani Kühn	631	Tulip poplar (collar rot)	.....	.....	J
Sclerotium bataticola Taub.	573	Red pine (collar lesion)	.....	Frederick Jc., Maryland	J

[a] Nomenclature after Wollenweber and Reinking (32).

[b] Isolations by: Y, Dr. H. H. York, Department of Botany, University of Penna.; E, E. J. Eliason, State Department of Conservation, Saratoga Springs, N. Y.; K, P. D. Keener, Dept. of Botany, University of Penna.; T, the writer; J, L. W. R. Jackson, B.P.I.S. & A.E., U. S. Dept. of Agriculture.

[c] Identified by C. D. Sherbakoff, Univ. of Tenn. Agricultural Experiment Station.

class thus included the decay of seed as well as the early rotting of radicles. (II) Post-emergence damping-off, the reduction of the total number of emerged seedlings through normal damping-off, top damping-off and root rot, all cases of infection being tabulated only if fatal before the termination of the experiment. The two categories could then be summarized in a survival count, based upon the number of seed planted, which, when contrasted with an uninoculated control, would provide a measure of the whole effect of the damping-off organism for the duration of the experiment.

MATERIALS AND METHODS

Isolations were made by dipping infected portions of diseased plants in four per cent formalin for two minutes and then placing them without rinsing upon two per cent agar without nutrients. A modification of this method was used with the fungi which were secured under conditions of pure saprophytism or whose origins were not known. Surface sterilized seeds of *Pinus resinosa* Ait. were sown on agar cultures of such fungi and then isolations were made from seedlings which damped-off after germination. With this method each fungus invariably produced a certain amount of injury, as demonstrated either by the decay of radicles or normal damping-off. Passing fungi of unknown pathogenicity through a potential host permitted the selection of more virulent strains, which could then be compared with other fungi isolated from authentic cases of the disease.

Monosporous transfers were made with a micromanipulator. Every monosporous culture coming from diseased seedlings, either directly by isolation or indirectly from passage through a host, is called a line throughout this paper. Stock cultures were maintained on potato-dextrose agar (Difco, pH 5.6) in cold storage at 8° to 10° C. or at room temperature from 18° to 25° C. A list of the names and sources of the fungi tested is given in table 1.

The selection of hosts for pathogenicity tests was based upon a compilation of susceptible conifers listed by Hartley (12). The list was restricted to abietinean conifers owing to their economic significance in reforestation and to the frequency of reports of disease among them. All seeds were kept in dry, unsealed containers in a cold-storage room at 8° to 10° C. A list of the species in the various inoculation tests is given in table 2.

Inoculations were made in liquid, quartz-sand, and autoclaved-soil cultures. All seeds, before planting, were surface-sterilized by immersion for 3 minutes in an aqueous solution of 0.1 per cent bichloride of mercury, followed by at least 5 rinses in sterilized distilled water.

Inoculum was prepared by growing the fungus on a substrate of 25 cc. of polished rice grains in 35 cc. of distilled water autoclaved for 20 minutes at 15 pounds' steam pressure. This sterile rice-mush was thoroughly permeated by the average *Fusarium* in 3 weeks at 28° C. Unless otherwise indicated, the inoculum was added to the substrate upon which the hosts were to be grown, at the approximate rate of one cubic centimeter per 5 square inches of surface. After the inoculum was mixed with the upper

inch of substrate, this entire layer was removed, thoroughly mixed in a mortar and replaced upon the surface of the pot or flat. This provided for uniform distribution of the inoculum in the upper inch of substrate, as well as for uniformity of age and quantity of inoculum of the different fungi. In the case of the liquid cultures, however, it was necessary only to add to the substrate a single hyphae-permeated rice grain of inoculum.

For those experiments in which liquid or quartz-sand media were used, a nutrient solution was selected which would be stable under emendation for pH and nutrition study and, at the same time, adequate for the growth of both hosts and pathogens. Glycerinated phosphates met these requirements. The advantages of substituting a salt of glycerophosphoric acid for that of phosphoric in standard nutrient solutions have been described (1). The solution ultimately adopted was a modification of a glycerophos-

TABLE 2.—*Seed source of conifers used in damping-off trials*

Species[a]	Year of collection	Locality of collection[b]
Pinus resinosa Ait.	1932	Adirondack region, N. Y. (a)
Pinus sylvestris L.	1938	Brushton CCC Camp, N. Y. (a)
Abies concolor Lindl. & Gord.	1940	San Isabel Forest area, Colorado (b)
Pinus nigra Arnold v. *austriaca* Aschers & Graebn.	1940	Illinois (b)
Picea pungens Engelm.	1940	San Isabel Forest area, Colorado (b)
Pinus ponderosa Dougl.	1940	Lewis and Clark N. F. Mont. (c) (4000 ft., kiln extracted)
Pinus banksiana Lamb.	1937–38	Superior, N. F., Minn. (c)
Pseudotsuga taxifolia Brit.	1939	Mt. Baker Forest, Darrington, Wash. (c)

[a] Taxonomy of conifers after Rehder (22).

[b] Seed received from: (a) E. J. Eliason, State Tree Nursery, New York State Conservation Department, Saratoga Springs, N. Y.; (b) Herbst Bros., New York City; (c) H. L. Shirley, Allegheny Forest Experiment Station, Phila., Pa.

phate type successfully used by Jackson (14) for the growth of Douglas Fir and *Pinus ponderosa,* as well as various isolates of *Pythium* and *Rhizoctonia.* The concentrations of the constituent salts were as follows: calcium nitrate, 0.007 M; magnesium sulphate, 0.04 M; sodium glycerophosphate, 0.04 M; potassium chloride, 0.01 M; iron citrate, to yield approximately 2 ppm. (1 ml. 1 per cent solution); boron (boric acid), 0.05 ppm.; copper (sulphate), 0.02 ppm.; manganese (chloride), 0.5 ppm.; and zinc (chloride), 0.05 ppm. The salts were made up in stock solutions, sterilized by autoclaving, and added to distilled water in the proper quantities to make the required concentrations as needed. The reactions of the solutions were adjusted to desired endpoints with 0.1 N HCl and 0.1 N NaOH. All pH determinations were made potentiometrically with quinhydrone and saturated calomel half-cells.

INOCULATION EXPERIMENTS AND RESULTS

Liquid Cultures

In preliminary tests, *Pinus resinosa* grown upon sterile liquid medium in test tubes was inoculated with a number of fungus lines (Fig. 1), following

with some modification the technique described by van Luijk (17) for grasses and applied to conifer study by Ten Houten (28). A hollow cylinder of filter paper, with the upper end covered by a lid bearing six 2-mm. holes

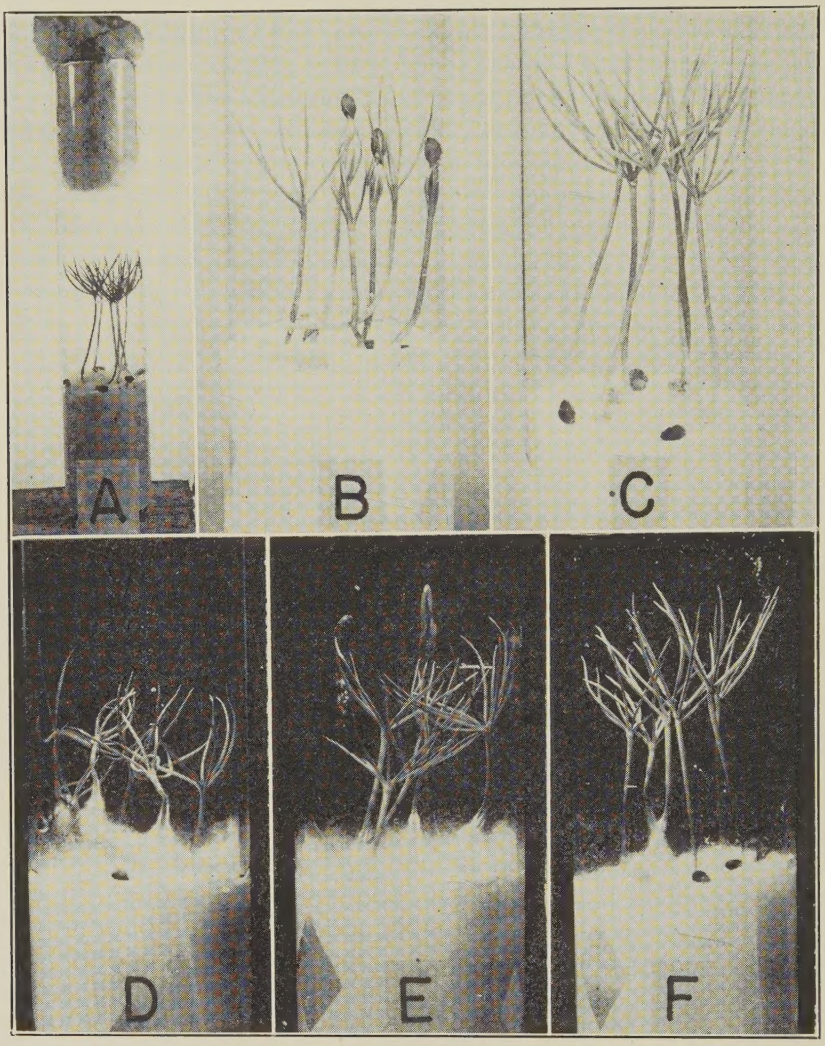

FIG. 1. Inoculation of seedlings of *Pinus resinosa* with lines of *Fusarium* in sterile liquid medium.

Control series: A. View of entire culture tube showing arrangement of seedlings. B. Enlarged view of sterile seedlings, 8 days after transplanting. C. Sterile seedlings 30 days after transplanting.

Inoculated series at termination of experiment (21 days). D. *Fusarium orthoceros* v. *longius* (R60), all seedlings attacked. E. *F. moniliforme* (R35), all attacked. F. *F. vasinfectum* (R34), 3 seedlings to right not attacked and without typical constrictions.

regularly distributed along the periphery and a larger aperture (5 mm.) in the center, was inserted into the bottom of a test tube (3.5 × 20 cm.). Exactly 50 cc. of nutrient solution, adjusted to yield pH 6 after sterilization,

was added so that the surface of the liquid reached the level of the lid. The tube was then plugged and autoclaved. Seedlings from surface-sterilized seed, germinated in sterile agar and closely checked for sterility after germination and with radicles from one to three cm. long, were transplanted to the tubes with aseptic precautions. The radicle of a seedling was inserted through one of the peripheral holes in the lid of the cylinder. The cultures were placed in a greenhouse (thermostatic control, 68°–75° F.) for three days. Seedlings that failed to grow were removed and replaced. When all seedlings were growing satisfactorily, the tube was inoculated by inserting a grain of inoculum into the liquid through the central hole in the lid. A small quantity of glass wool within the cylinder served to lodge the inoculum

TABLE 3.—*Damping-off of Pinus resinosa seedlings inoculated with various fungi in liquid cultures*

Line no.	Fungus	Number of seedlings inoculated[a]	Number of seedlings invaded	
			After 10 days	After 21 days
R11C	*Fusarium poae*	12	12	12
R34	*F. vasinfectum*	18	10	14
R33	*F. sporotrichioides*	36	27	35
R60	*F. orthoceros* v. *longius*	18	12	18
16	*F. orthoceros*	18	17	18
64	*F. avenaceum*	12	12	12
R32	*F. javanicum v. radicicola*	18	6	9
58	*F. reticulatum*	12	12	12
1C	*F. aquaeductuum* v. *medium*	12	3	7
R8A	*F. sambucinum*	18	5	7
R35	*F. moniliforme*	18	11	18
R2A	*F. solani* v. *martii* f. 1	12	3	8
631	*Rhizoctonia solani*	12	12	12
Control		24[b]	0	0

[a] Six seedlings per tube.

[b] An additional control tube showed a mold several days after the seedlings were transplanted. Two seedlings were rotted at the expiration of the test (21 days).

just below the surface of the liquid, as well as to provide support for the seedlings. Holes cut in the sides of the cylinder permitted the observation of the development of the host and the fungus. The inoculated tubes were kept for 3 weeks in the greenhouse.

The criterion of whether a fungus was able to attack the host was the visible evidence of a constriction in the root or hypocotyl, where the cortical tissue was destroyed. A collapse of the seedling, the typical normal damping-off symptom, could not always be used because the seedling received considerable support from adjacent plants and from the wall of the tube; nor could fungal growth along aerial portions of the plant be used as a sign of infection, since this commonly occurred because of high humidity. However, from seedlings with typical constrictions (Fig. 1, D, E and F), surface-sterilized and sown on potato-dextrose agar, the inoculated fungus could be isolated. Plates from unconstricted seedlings, with the same treatment, invariably remained sterile.

All 12 of the Fusaria in the liquid-culture inoculation tests were pathogenic (Table 3). *Fusarium avenaceum, F. poae, F. moniliforme, F. reticulatum* and the two varieties of *F. orthoceros* caused complete losses, comparing thus in virulence with the Rhizoctonia line. *F. sporotrichioides* was also highly parasitic. The rest of the lines were moderately parasitic. *F. sambucinum* was the least virulent of the lines tested in this experiment.

Quartz-Sand Cultures

The quartz-sand cultures (Fig. 2) were maintained in four-inch pots with sand that had been washed for several hours in cold, running water, soaked in hot water, and then rinsed in distilled water. Each pot was painted on all surfaces with DuPont acid- and alkali-resistant black paint to

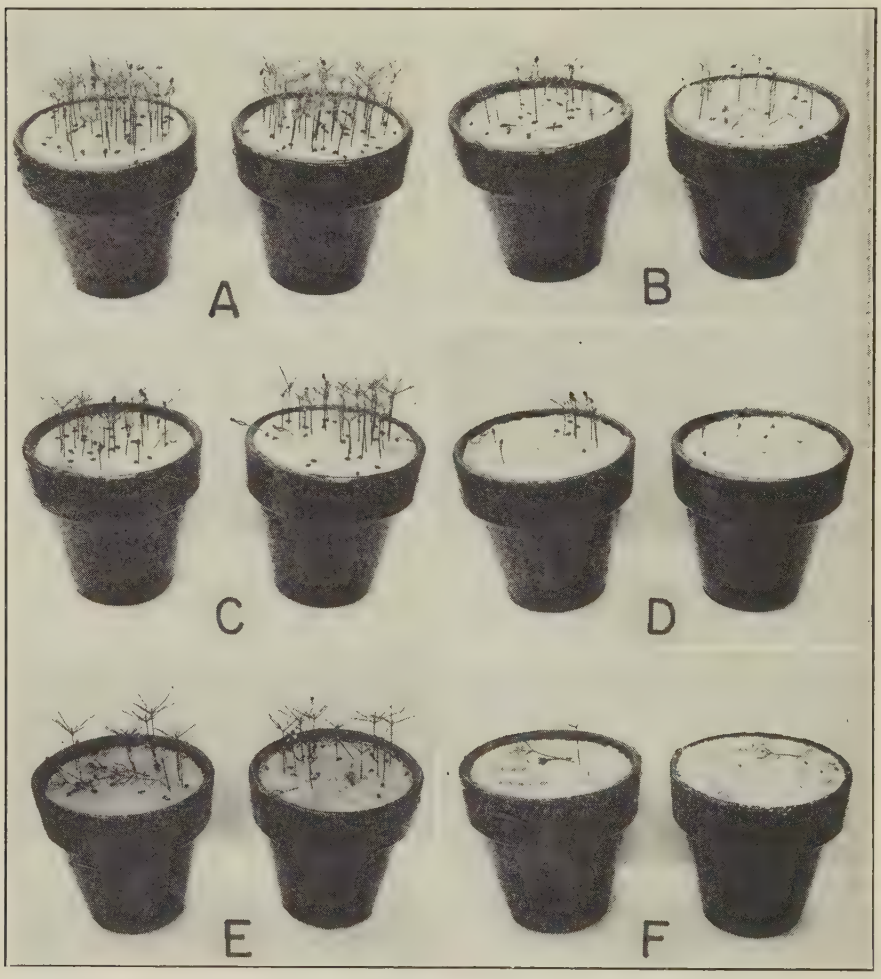

FIG. 2. Inoculations in quartz-sand cultures of *Pinus resinosa* with lines of *Fusarium*, photographed 26 days after planting and 14 days after emergence: A. Controls. B. *F. orthoceros* v. *longius* (Line 61). C. *F. poae* (Line 68). D. *F. reticulatum* (Line 58). E. *F. oxysporum* (Line 30). F. *F. avenaceum* (Line 66).

reduce water loss through evaporation. The pots of sand were covered with Petri-dish lids and autoclaved for one hour at 15 pounds. After inoculation, the pots were flooded with the standard nutrient solution adjusted to pH 6, and were incubated at room temperature for several days to permit thorough permeation of the sand by the fungi. Each line was inoculated in duplicate and controls were prepared with sterile rice-mush. Fifty surface-sterilized

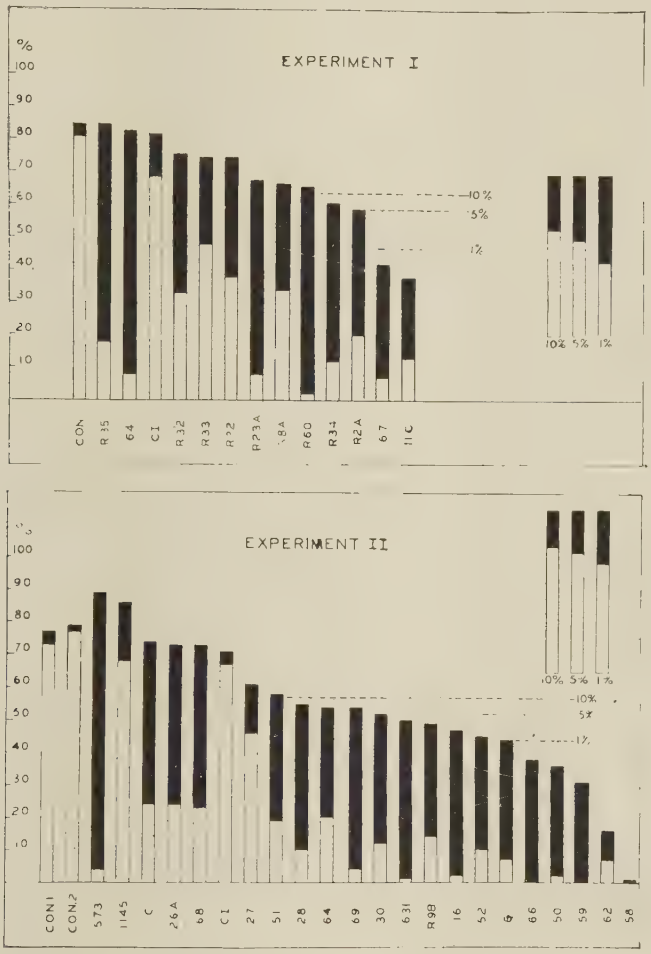

FIG. 3. Diagram showing the results of inoculations of quartz-sand cultures of *Pinus resinosa* with various fungi: comparative emergence (length of bar), post-emergence damping-off (black) and final stand (white). Levels of significance for emergence (dashed lines) and post-emergence losses (black portions of bars to right) are given.

seeds of *Pinus resinosa* were then sown on the surface of each pot and covered with one-quarter inch of sterile sand. The pots were kept in the greenhouse and watered daily by flooding with nutrient solution freshly prepared by adding sterile stocks to distilled water, without autoclaving. The glass covers were removed after the seedlings began to emerge from the sand.

All the fungus lines were tested in two experiments, and although the

first was started two weeks before the second, each was maintained for three months under identical greenhouse conditions. The seedlings which damped-off after emergence were removed as soon as recognized and the numbers recorded. The results are in figure 3. The error variance, or the differences in results derived from each of a pair of duplicate pots in a treatment, was statistically employed for determining the significance of the variance between lines and between each line and the control used as a standard (18, 23).

A certain amount of post-emergence loss was evident in all the control series, although relatively small. This was unavoidable, since no precautions were taken to prevent contaminations by air-borne organisms, and in the duration of the experiment some managed to enter the controls. Fusaria were isolated less than once out of eight times from control seedlings which damped off.

Sterilized-Soil Cultures

Inoculation tests in soil were performed on all the coniferous hosts with a selection of Fusarium lines and the fungi used as standards (Fig. 4). The soil contained three parts of loam, two of sand, and two of leaf-mold. After steam sterilization the final pH was 5.8. The soil was placed in wooden flats, with inside dimensions of $9 \times 12 \times 5$ inches and each was autoclaved for one hour at 15 pounds pressure on two successive days. Following sterilization, heavy paper covers reduced air contamination until seedlings began to emerge, after which the covers were removed. Flats were inoculated, and controls were prepared, as were pots for the sand-culture experiments. The general thinning of stands (Fig. 4), as compared with the control, indicated a uniform distribution of inoculum.

Three rows of 50 or 60 seeds of each host, depending upon the relative size of the seed, were sown in each flat, making the density of sowing in soil comparable with that in the sand experiments. Each flat was watered daily by top-irrigation with distilled water. The tests were maintained for four months in the greenhouse (daily mean temperature, 68–80° F.), after which the surviving seedlings were removed, the flats resterilized and the entire set of inoculations repeated. Thus the duplicate set in this case was not run concurrently. However, the greenhouse conditions under which both sets were maintained, the first in the spring and early summer and the second in the fall of that year, were made as closely alike as possible and there was no variation in the standard inoculation and planting procedures. The results of the inoculations are given quantitatively in figure 5, each percentage value being the average of the two trials. Some damping-off again occurred in the controls, but as in previous experiments, only a relatively small percentage of the isolations from these seedlings yielded Fusarium species. The losses caused by this accidental contamination were extremely small and insignificant in comparison with those in the inoculated flats.

No attempt was made to evaluate the virulence of the various fungi on the basis of a consideration of each host-fungus combination. As is evident

from the results, most of the lines showed variability in virulence within themselves on the different conifers. Some lines were fairly consistent: for instance, *Fusarium poae* (Line 11C) and *F. reticulatum* (Line 58) repeatedly reduced emergence of all the hosts to 10 per cent or less of seed sown;

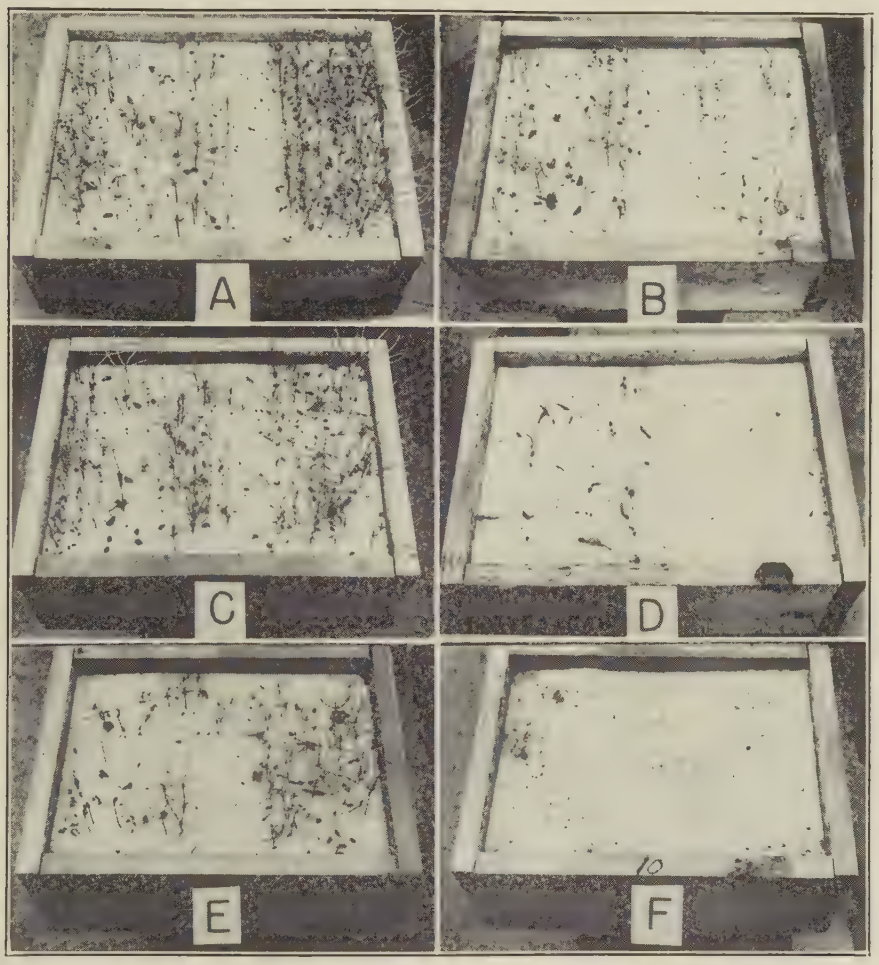

FIG. 4. Inoculations in autoclaved-soil cultures of coniferous hosts with various fungus lines. Hosts arranged in groups of three rows, left to right: *Pinus resinosa, Abies concolor, Picea pungens, Pinus ponderosa, Pinus banksiana,* blank (originally planted with seeds of *Pinus strobus* L. but seeds germinated too late and in too few numbers to be comparable with other hosts), *Pinus sylvestris, Pseudotsuga taxifolia* and *Pinus nigra austriaca.* A. Control, 28 days after planting. B. *F. equiseti* (Line 27), 22 days. C. *Sclerotium bataticola* (Line 573), 28 days. D. *F. avenaceum* (Line 66), 20 days. E. *F. orthoceros* (Line 23A), 26 days. F. *F. reticulatum* (Line 58), 23 days.

while *F. avenaceum* caused severe or complete post-emergence losses of all the hosts. Another means of analyzing the data was to consider the pathogenicity of each fungus on all the hosts simultaneously, expressed in terms of the mean emergence or mean post-emergence damping-off that each caused

on all the conifers. Conversely, from the same data, it was possible to measure the relative susceptibility of each host to all the fungi and express it as

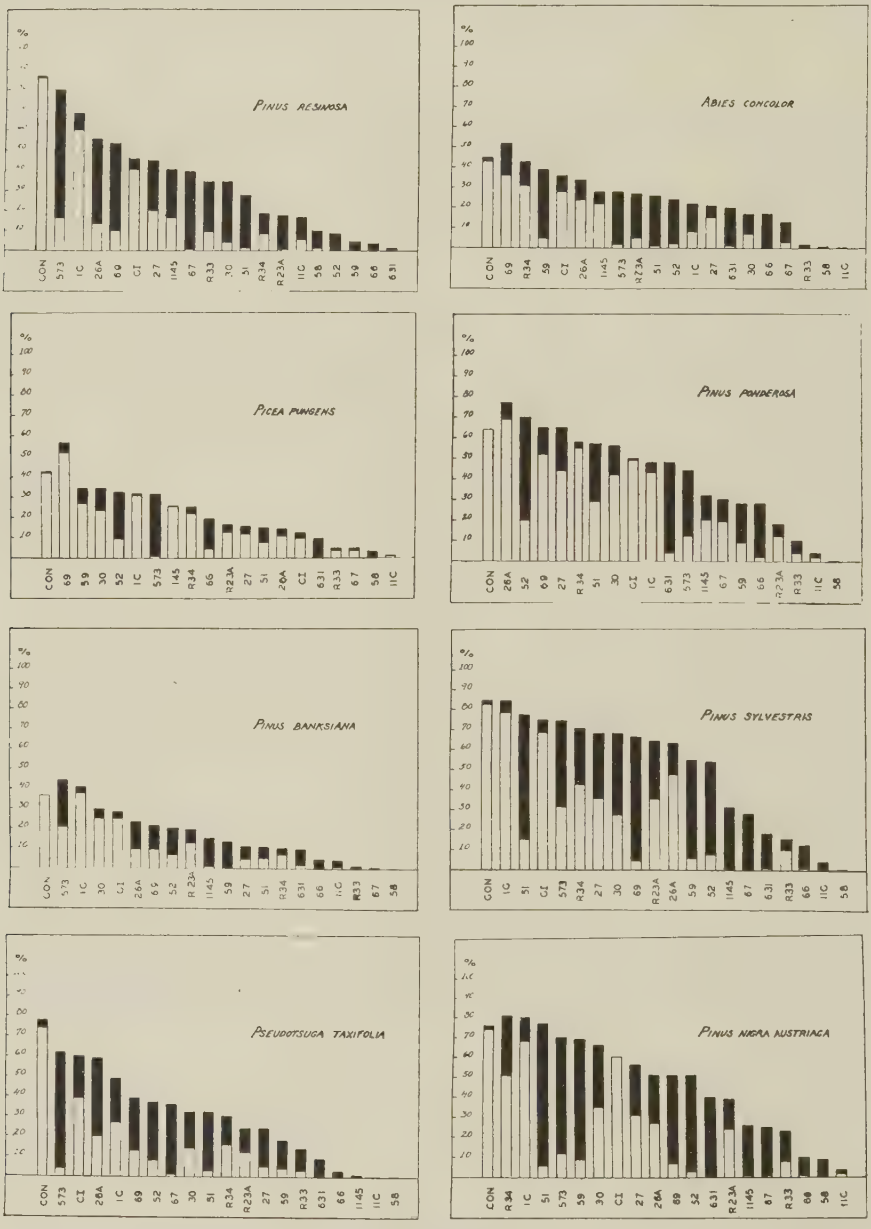

FIG. 5. Diagram showing results of inoculations with various fungi in sterilized-soil cultures of conifers: emergence (Length of bar); post-emergence damping-off (black); final stand in percentage of seed (white).

a mean value. The significance of the differences between such means was determined by analyses of variance, subdividing the variance into three

TABLE 4.—*Total percentage emergence of all hosts in autoclaved-soil cultures inoculated with various fungus lines*

Fungus line	Average percentage emergence of all hosts for each fungus line		Order of ability to reduce emergence
	Actual means	Means based upon controls evaluated at 100 per cent	
58	2.9	4.2	1
11C	4.1	6.2	1
R33	13.2	17.6	2
66	12.4	22.3	3
67	22.2	31.2	4
631	19.6	32.4	4
1145	24.9	41.7	5
R23A	28.3	45.2	6
27	35.7	53.1	7
59	32.6	53.7	7
52	40.3	59.9	8
R34	42.0	65.9	9
30	42.2	67.5	9
CI	46.0	69.9	10
26A	47.5	72.1	11
1C	53.1	82.8	12
69	50.7	83.5	12
573	54.4	84.8	12
Con	64.2	100.0	
Least significant difference between means	1.3	2.2	

portions: among fungi, among hosts, and between duplicate series of tests (18, 23).

These analyses revealed extremely high variability among fungi and among hosts. In considering emergence data, a slight significance was apparent in the variance due to duplication; however, upon evaluating the emergence in the controls for each series at 100 per cent and reanalyzing the emergence in the inoculated flats in terms of percentage of control, the significance for duplicate trials disappeared. Thus the general effects of the

TABLE 5.—*Total percentage emergence of each host in autoclaved-soil cultures inoculated with various fungus lines*

Host	Average percentage emergence of hosts for fungus lines		Order of susceptibility to emergence loss by Fusaria
	All lines	Fusarium lines	
Pinus resinosa	36.5	33.3	1
Pseudotsuga taxifolia	35.9	34.4	1–2
Pinus banksiana	44.5	38.3	2
Picea pungens	48.2	48.6	3
Abies concolor	53.0	50.6	3
Pinus sylvestris	57.4	56.8	4
P. nigra austriaca	61.7	60.8	4–5
P. ponderosa	63.9	62.9	5
Least significant difference between means of all lines		4.6	

TABLE 6.—*Total percentage post-emergence damping-off of all hosts in autoclaved-soil cultures inoculated with various fungus lines*

Fungus line	Average percentage post-emergence loss of all hosts for each fungus line	Order of ability to cause post-emergence loss
631	95.8	1
66	94.0	1
52	83.8	2
67	82.4	2
573	78.2	3
59	77.6	3
51	76.4	3
R33	64.2	4
1145	58.5	5
69	55.9	5–6
R23A	52.8	6–7
30	50.5	7
27	44.8	8
26A	39.9	9
R34	34.4	10
1C	21.6	11
CI	13.5	12
Con	2.2	.....
Least significant difference between means	3.7	

fungi in the two trials were fundamentally alike; the general emergence potential differed, a condition probably arising from unavoidable variations in the extremely critical sterilization treatment of the seeds. The assumption of optimum germination in the controls permitted a comparison of host reactions to the fungi apart from the different capacities of the seed for germination.

On the basis of these analyses, the results of the soil-culture experiments could be arranged in orderly sequences. The average percentages of emergence of all the hosts for each fungus treatment are given in table 4 in order of increasing sprouting, and the actual means as well as those based on con-

TABLE 7.—*Total percentage post-emergence damping-off of each host in autoclaved-soil cultures inoculated with various fungus lines*

Host	Average percentage post-emergence loss of hosts for fungus lines		Order of susceptibility to post-emergence loss by Fusaria
	All lines	Fusarium lines	
Pinus resinosa	65.8	75.9	1
Pseudotsuga taxifolia	69.9	73.4	1
Pinus nigra austriaca	62.8	65.2	2
Abies concolor	59.4	65.1	2
Pinus sylvestris	57.0	58.6	3
P. banksiana	51.1	52.3	4
P. ponderosa	39.7	39.1	5
Picea pungens	35.7	31.5	6
Least significant difference between means of all lines	5.6		

trols equal to 100 per cent emergence are included. In addition, the lines are ranked on the basis of their ability to reduce emergence. In table 5 is a like arrangement of hosts, based upon the average emergence after inoculations with all fungus lines, and with Fusarium lines alone. The hosts were ranked in order of susceptibility to emergence loss from the Fusaria. Similar rankings with respect to post-emergence losses are in tables 6 and 7.

DISCUSSION

The results of these inoculation experiments with various lines of fungi, predominately Fusaria of parasitic and saprophytic origin, on a general coniferous population, indicated that certain species of Fusarium were capable of causing extreme reductions in emergence of the total viable seeds of the hosts (Figs. 3 and 5). In some cases the losses were greater than those caused by the standard lines of *Rhizoctonia, Sclerotium,* and *Pythium.* Furthermore, some Fusarium lines were comparable with these standards in their ability to cause post-emergence damping-off. In the two sand-culture experiments, 15 of 29 different Fusarium lines tested, significantly reduced emergence in comparison with the respective control series, and 8 of these were beyond the 1 per cent level of probability. Three additional lines produced reductions of questionable significance. All the lines save *F. equiseti* caused significant post-emergence losses, the exception occurring within the doubtful probability level. Of the standard lines, a common mold, *Penicillium* sp., and the *Pythium* line, in both experiments neither reduced emergence nor caused significant post-emergence loss. *Sclerotium bataticola* produced no emergence loss but did cause a high percentage of post-emergence damping-off, and *Rhizoctonia solani* was highly virulent both before and after seedling emergence.

Different isolates within the species *Fusarium orthoceros, F. reticulatum, F. avenaceum,* and *F. poae,* varied in ability to cause emergence losses in conifer seedlings.

In the soil experiments all the lines tested significantly reduced emergence of the hosts when compared with uninfected controls. Five lines of *Fusarium* caused greater emergence losses than did the lines of *Rhizoctonia* or *Pythium.* Six additional Fusaria ranged in virulence from a degree comparable with these standards to one resembling the *Penicillium. Sclerotium bataticola* reduced emergence the least of all the lines tested. All lines caused significant post-emergence losses when compared with the control. *Rhizoctonia solani* and *Fusarium avenaceum* caused the greatest losses. From the high rate of mortality of the few seedlings which germinated in inoculations with *Fusarium poae* (Line R11C) and *F. reticulatum* (Line 58), these lines appeared capable of inducing post-emergence losses comparable with emergence losses; however, for reasons already stated, these were omitted from the analysis. Three Fusarium lines were more virulent than the *Sclerotium,* and six were more virulent than the *Pythium. F. aquaeductuum* v. *medium* caused the least damage of the Fusaria, and the Penicillium less than any of the lines.

Failures of emergence in coniferous nursery beds have long been associated with damping-off organisms; however, species of *Fusarium* on the whole have generally been credited with less ability to cause pre-emergence than post-emergence losses (11, 12, 13, 28). Results of the present experiments indicate relatively equal virulence for Fusaria in both aspects of this disease, and agree with other observations (8, 21) that many Fusaria may reduce sprouting by the early decay of radicles. One author has stated that *Pythium ultimum* and virulent strains of *Corticium* (*Rhizoctonia*) *solani* apparently caused more germination losses of conifers than any Fusarium line tested, with the exception of *F. sporotrichioides* (21). The present investigations in soil and sand substrates, particularly the latter, indicate to the contrary that a number of Fusarium lines may reduce emergence to a greater extent than the species mentioned.

Rather high emergence losses and post-emergence losses consistently resulted from inoculations with *Fusarium reticulatum* (Line 58), *F. poae* (Line R11C), *F. avenaceum* and *F. sporotrichioides;* moderate losses of both types followed inoculations with *F. vasinfectum* and *F. oxysporum;* but *F. aquaeductuum* v. *medium* caused little loss of either kind. Other lines were less consistent: some caused considerable emergence reduction with relatively little subsequent attack; others reduced emergence comparatively little, but caused high post-emergence losses.

The virulence of the fungi depended to a very large extent upon the conditions under which they were tested. In liquid culture inoculations in test tubes, where only post-emergence attack was considered, all the lines caused appreciable or complete destruction of seedlings, even the relatively nonvirulent Line 1C. The conditions set up favored the fungus to such extent that even the least virulent organism attacked the host in the unnatural environment. Fine distinctions of relative virulence are not obtained by this method. In quartz-sand cultures, where the host had a solid substrate, approximately half the lines significantly reduced emergence while almost all caused heavy post-emergence losses. Here again conditions favored the fungi to the extent that the severity of most of the losses permitted little comparison of relative virulence of the fungi.

The soil-culture method, wherein most of the lines caused both types of loss, represents the closest approach in the laboratory to a duplication of nursery conditions. However, some artificiality is again evident, particularly in the sterilization of the soil, a procedure which is essential in order to remove potential damping-off organisms or any others that might affect the activity of the test organism, but which, as shown by Lindfors (16), unquestionably increases the severity of Fusarium attack. The generally greater emergence in the sand cultures compared to that in soil could not be explained as inability of the fungus to thrive in the sand, since sufficient rice-mush was carried along and uniformly distributed with the fungus mycelium in the process of inoculation so that ample nutrition was afforded the fungi. In addition, the fungi received accessory nutrient material in

the sand cultures from the standard nutrient solutions supplied. The poorer retention of moisture by the coarse particles of sand, with consequently better aeration, probably stimulated emergence and allowed the fungi less opportunity to act upon radicles. These results paralleled Spaulding's observation (27) that the use of a coarse sand cover apparently increased resistance to the disease.

The simultaneous inoculation of all hosts as in the sterilized-soil tests, permitted a valid comparison of their relative susceptibility to Fusaria. Emergence and post-emergence losses were greatest in *Pinus resinosa* and *Pseudotsuga taxifolia*. Hartley's (12) observations have indicated, somewhat differently, that *Pinus resinosa* is more susceptible to the later forms of damping-off than to germination losses. However, from his compilation of the literature on relative susceptibility of conifers, there is general agreement with the present results for *P. resinosa,* most of the reports indicating a relatively high degree of susceptibility to damping-off. *Pseudotsuga taxifolia* frequently has been reported as somewhat less susceptible, although later work (5) describes severe attacks on this species. Rathbun-Gravatt (21) had comparable results on *P. resinosa* and *P. banksiana,* the seeds of the former showing reduced germination percentages in the presence of Fusarium, a result compatible with those of the present investigation. *P. banksiana* proved somewhat less susceptible to post-emergence losses. *Abies concolor* was of moderate relative susceptibility to both categories of losses, somewhat at variance with early reports of its extremely high susceptibility to damping-off (3, 4). Hartley (12) states that literature reports indicate relative susceptibility of *Pinus ponderosa* and resistance of *Picea pungens.* The present results agree with the latter observation. *P. pungens,* although moderately attacked before emergence, suffered the least post-emergence loss of the conifers tested. *Pinus sylvestris* has variously been reported as ranging from less susceptible than average to most susceptible (12). The present tests have indicated its relatively intermediate response to both types of attack.

In all these experiments, the virulence of the fungi probably depended to a considerable extent upon the nature of their nutrition prior to inoculation. Fusaria grown upon rice decoction have been more virulent than the same organisms from nutrient agars (20, 28). This is probably due to a higher production by the fungus of toxic materials on the rice, as suggested by the investigations of Ten Houten (28) and of van Eek (7), who is cited by the former to have secured a stronger attack with the extract of a Fusarium culture on rice than with the living fungus itself. Hence, in all the experimental methods used in these studies, the employment of moderately heavy, toxic inoculum, dense sowing, abundant moisture, and greenhouse conditions unquestionably favored the development of the fungi to an extent far exceeding that which would normally be encountered in the field; and probably the magnitude of the losses seen in the tests would ordinarily not

be met in the nursery. However, the possibility remains that under some circumstances, conditions might become similarly favorable for the establishment of comparable host-pathogen relationships. Thus species of *Fusarium,* normal saprophytes in the nursery, might suddenly become highly virulent.

SUMMARY

Four fungi used as standards and monosporous cultures of twenty-five Fusarium lines, some parasitic and others saprophytic, were tested for ability to cause damping-off of coniferous species. A method of host-passage to determine pathogenicity is described. Eight coniferous hosts were selected for inoculation tests.

Losses are tabulated as: (I) emergence loss, including seed decay and destruction of radicles before emergence of seedlings from the substrate; and (II) post-emergence loss, including normal damping-off, top damping-off, and all root-rot fatal before the termination of the experiment.

The relative values of some standard inoculation procedures for testing relative virulence is discussed.

In liquid-culture inoculation tests in glass tubes, of twelve lines of *Fusarium* tested on seedlings of *Pinus resinosa,* six caused complete loss, comparing in virulence with a standard line of *Rhizoctonia solani.*

All of the lines were tested in quartz-sand cultures of *Pinus resinosa* seedlings. Approximately half of the Fusaria reduced emergence significantly compared with the uninfected control, and all save one caused heavy post-emergence losses, the significance of the exception being questionable. Of the standard lines: *Sclerotium bataticola* did not lower emergence but caused high post-emergence losses; *Rhizoctonia solani* effected severe losses in both categories; and *Pythium ultimum* and *Penicillium* sp. did not significantly reduce emergence or cause post-emergence losses.

Inoculation tests in sterilized-soil culture gave the most statistically reliable results of the methods employed because of the higher degree of replication available for analysis. The relative virulence of fifteen Fusarium lines and four standard cultures for a general coniferous population, represented by the eight hosts, was determined; and conversely, the range of susceptibility of the hosts to the entire group of damping-off fungi was ascertained. A number of the Fusarium lines equalled and occasionally exceeded in virulence the cultures of *Pythium* and *Rhizoctonia,* causing reduced emergence and increased post-emergence losses. *Sclerotium bataticola* caused the least emergence loss but post-emergence attack was relatively high. *Pinus resinosa* and *Pseudotsuga taxifolia* were equally most susceptible to both phases of the disease. *Pinus ponderosa* had the least emergence loss and *Picea pungens* the least post-emergence loss.

Nursery losses due to these fungi could be expected to be less severe than those thus experimentally induced; however, conditions under some circumstances might resemble those in the tests, in which event losses of similar

magnitude caused by normally saprophytic or moderately parasitic species of *Fusarium* could be expected.

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STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. II. RELATION OF AGE OF HOST, pH, AND SOME NUTRITIONAL FACTORS TO THE PATHOGENICITY OF FUSARIUM[1]

HOWARD TINT[2,3]

(Accepted for publication January 15, 1945)

The damping-off diseases of coniferous seedlings are usually epidemic in character. Their economic significance is due to the irregularity of the losses as well as to the severe reductions in nursery stands. In a single nursery, in one season, damage may be negligible or moderate; whereas, in the next growing period, losses may be severe. Adjacent seed beds may be attacked in highly varying degrees. Obviously, certain predisposing factors must operate, singly or in a particular combination, at any given time, to account for the variability in losses.

Under certain conditions many species of *Fusarium* cause severe reductions in emergence as well as high post-emergent mortality (36). The present investigation deals with variations in environmental conditions affecting this host-pathogen relationship.

GENERAL MATERIALS AND METHODS

The selection of hosts and damping-off fungi was based upon results from a previous experiment (36). The sources of seeds and of the Fusarium lines were described in detail, as well as standard methods of seed sterilization, selection of monosporous cultures of virulent strains of fungi, preparation of inoculum and glycerophosphate nutrient solutions for the hosts and fungi. A division of the damping-off losses into two categories, emergence loss and post-emergence damping-off has been described. The same division was followed in evaluating losses due to inoculations with fungi in the following tests. Where experimental procedure required occasional departures from these standard methods, the alternative treatments are described.

All seedlings were grown in a low-humidity greenhouse, thermostatically adjusted to maintain a daily mean temperature range of 68–75° F., in those seasons of the year when such control was possible.

Determinations of pH were made potentiometrically with quinhydrone and saturated calomel half-cells, and all solutions were adjusted in acidity with 0.1 N HCl and 0.1 N NaOH.

AGE OF HOST

Post-emergence losses from damping-off generally diminish with in-

[1] This paper constitutes one section of a thesis submitted to the faculty of the Graduate School of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

[3] The author wishes to express his appreciation to Dr. W. A. Campbell and Dr. B. Sleeth for valuable suggestions regarding the preparation of the manuscript.

crease in age of coniferous seedlings (10, 11). Early losses have been associated with the tenderness of the young seedlings. With the development of woodiness in the hosts, ordinary damping-off losses became relatively negligible. Early post-emergence attacks, invariably fatal, have been included in the category of ordinary or normal damping-off losses. Those occurring after this initial period, and not necessarily fatal, have been described as root-rot. Emphasis of the present studies has been placed primarily on the former type, and an attempt was made to determine how long after emergence seedlings were subject to normal damping-off attack by Fusarium species.

Initial observations on the relation between age of host and susceptibility to attack were made upon seedlings of *Pinus resinosa* grown in quartz-sand

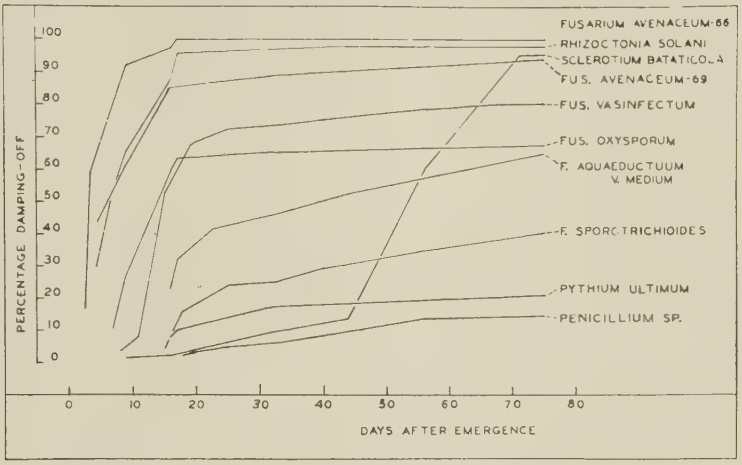

FIG. 1. Relation of time to post-emergence damping-off of *Pinus resinosa* seedlings inoculated in quartz-sand cultures with Fusarium and other standard fungus lines.

cultures, watered daily with the standard nutrient solution adjusted to pH 6. These were in 4-inch pots that had been inoculated with Fusarium or other fungus lines prior to the planting of fifty seeds in each. Periodic records were made of the losses that occurred after the emergence of the seedlings. The damping-off loss in each culture was recorded as the percentage of the total number of seedlings that had emerged up to that time.

The results are in figure 1, the curves being based upon the averages of duplicate series for each fungus line. On seedlings in contact with the more virulent fungi, effects of attack were visible soon after emergence, and maximum losses were reached within approximately three weeks. The effects of less virulent fungus lines did not become noticeable until later, although maximum losses developed before 30 days. For all of the lines there were slight increases in damping-off with time, following the initial surge of attack. Losses from *Sclerotium bataticola* were low for the first six weeks but became very pronounced thereafter, reaching an extremely high level at the termination of the experiment. This tendency for *Sclerotium* to be only

slightly effective in early attack, in comparison with *Fusarium sp.*, has already been noted (36).

In order to verify that these variations in host-pathogen relationship were due to increasing resistance of the host with age, healthy seedlings of *Pinus resinosa* of varying ages were transplanted to pots of nutrient-watered sand, that had previously been inoculated with rice cultures of *Fusarium oxysporum* and incubated at room temperature for five days. Younger seedlings were taken from a sterile germinator, whereas older ones were grown in uninfected quartz-sand in the greenhouse. Each age group was tested in duplicate, at the rate of 40 seedlings per pot, and losses due to damping-off were recorded for 30 days after transplanting. Losses in the cultures, which were run simultaneously in the greenhouse, are given in figure 2.

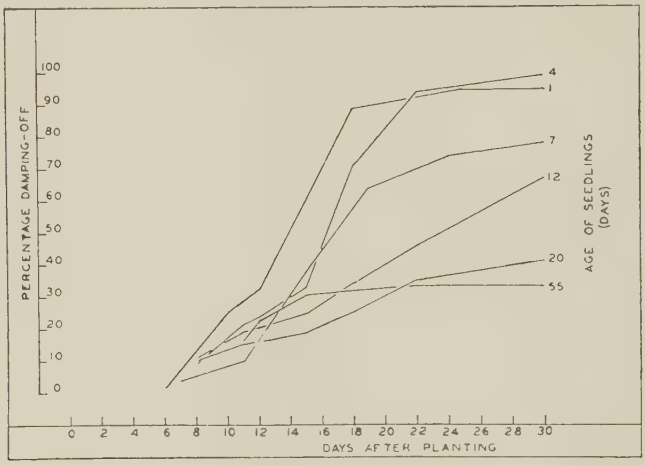

FIG. 2. Relation of age of seedlings of *Pinus resinosa* to rate and total extent of damping-off in quartz-sand cultures inoculated with *Fusarium oxysporum*.

Seedlings from 1 to 7 days old had the higher percentage of damping-off, and the reduction in levels of losses corresponded with the increasing age of the host. The time between initial exposure of the seedlings to the fungus and the first symptoms, varied from 6 days with the one-day-old seedlings to 11 days for the 55-day-old plants.

pH STUDIES

Soil reaction may affect forest-tree seedlings directly through the influence of hydrogen or hydroxyl ions, or indirectly by influencing the availability of nutrients, the physical condition of the soil, and the activity of parasitic and saprophytic soil-organisms. Conifers, in general, prefer an acid reaction for optimum seed germination (1, 4, 5, 6, 31, 37, 39) and for subsequent growth and development (6, 14, 38, 40). Species of *Fusarium*, in the laboratory, have a wide tolerance for variations in the reaction of substrates, ranging from approximately pH 2 to 11, but optimum growth has usually been observed on media of acid or nearly neutral reaction (17, 19, 20, 27).

Early observations by Gifford (9) revealed that an increase in the alkalinity of the soil by liming increased the amount of subsequent damping-off, whereas manuring treatments which tended to keep the soil reaction acid, reduced the damage. Anderson (3) observed that the application of wood ashes to seed beds before sowing increased damping-off of seedlings of *Pinus sylvestris* and *Picea sitchensis*. Hartley (10, 12) observed that damping-off in forest nurseries was not serious in soils with a pH lower than 6. In addition, soil acidification treatments have been used to control the disease (7, 10, 12, 18, 33, 35), although with only partial success in some localities.

These observations have to a large extent been confirmed by inoculation tests. Roth's investigations (29) on *Picea excelsa* inoculated in soil cultures with *Pythium deBaryanum* and *Rhizoctonia solani,* revealed no serious losses at pH values below 5.5, maximum attack on the acid side of neutrality, and again a lessening of losses at pH values above 7. The last effect is at variance with general experience in the field, where heaviest losses occur at an approximate pH value of 8 (10). Inoculations of Douglas Fir and ponderosa pine with isolates of *Pythium* and *Rhizoctonia* by Jackson (16), gave similar results. In sand cultures, however, Jackson's results showed better agreement with field experience for the higher pH values, although somewhat more damping-off at the acid levels was obtained than under natural conditions. Ten Houten (35), from inoculation tests on *Pinus nigra austriaca,* in soils that varied in pH and in organic matter, concluded that the parasitism of *Pythium deBaryanum, Rhizoctonia solani,* and *Fusarium oxysporum,* was highly dependent on the reaction of the soil. In those soils in which the pH was 4.5 or lower, the damage caused by these fungi was not important.

A preliminary test of the relationship of pH and soil-sterilization to damping-off was performed in soil samples artificially adjusted into a pH series according to a method used by Roth (29). A soil was prepared from three parts of loam, two of sand, and one each of leaf-mold humus and acid peat (Canada). To half of this mixture one extra part of peat was added and to the other one part of humus, yielding respective pH values of 5.1 and 6.3. By the addition of graded amounts of coarse sodium-calcium hydroxide (40 per cent NaOH, 57 per cent $Ca(OH)_2$, 2 per cent H_2O) to the more alkaline portion, samples with pH values of 6.9, 7.9, 8.3, and 8.6 were obtained. Similar treatment of the acid portion with sodium bisulphate (35–36.5 per cent H_2SO_4) yielded lots of pH 4.9 and 3.6. A mixture of the two original lots gave a sample of pH 5.6. In preliminary investigation, soil samples similarly adjusted maintained their new reactions rather closely for a long time if continually moistened with water adjusted to corresponding acidity levels. All acidity determinations, however, were made upon samples air-dried in the laboratory for several days. This method of adjusting and maintaining the acidity of soil provided samples highly comparable in physical and basic chemical content, differing mainly in respect to the acidifying and alkalizing substances added. It permitted a more significant compari-

son of the effects of the reactions on the growth of the host and the damping-off losses induced by inoculated fungi than would have been possible in a collection of soil samples from different localities.

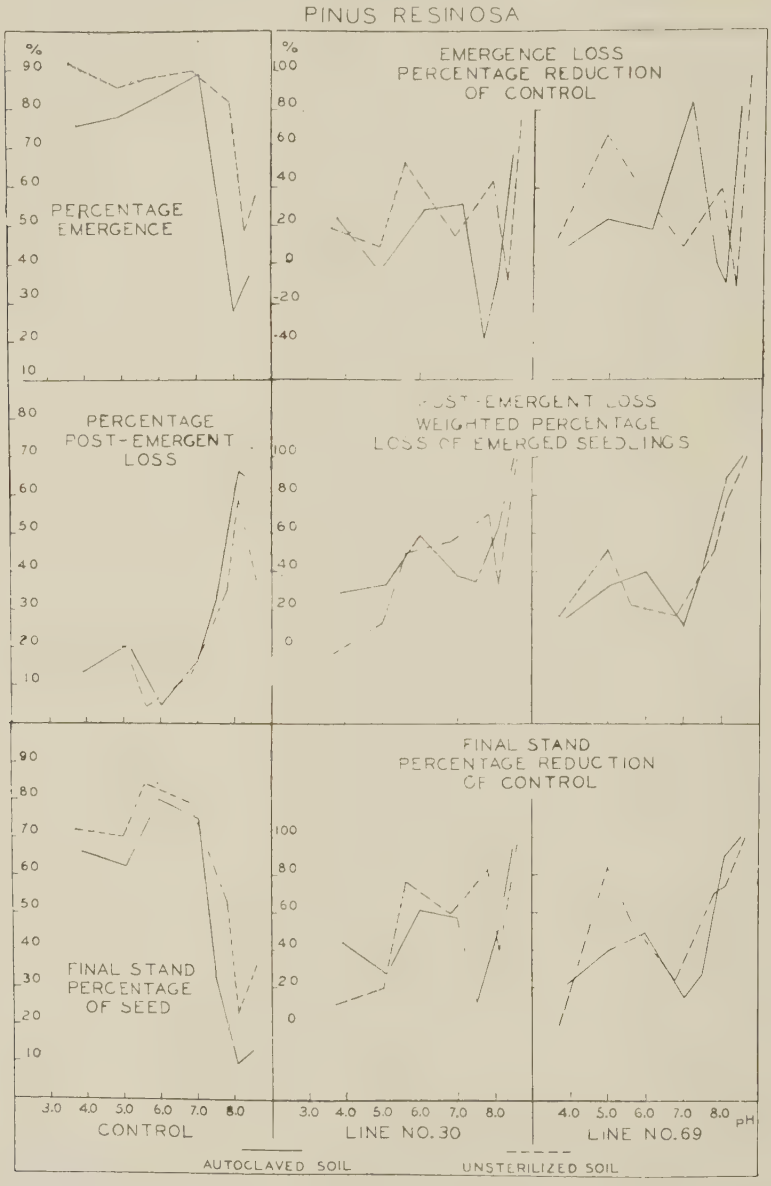

FIG. 3. Relation of pH to the growth and damping-off of *Pinus resinosa* in soil cultures inoculated with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results for inoculated series expressed in terms of corresponding control data.

Each pH lot was distributed into twelve 7-inch pots, and six of each group were autoclaved at 15 pounds for one hour on two successive days. The resultant pH values in the autoclaved samples were 3.8, 4.9, 6.1, 7.1, 7.7,

8.0, and 8.4. Duplicate pots were then inoculated with rice-mush cultures of *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69), sterile rice medium being added to the control series. After incubation for one week

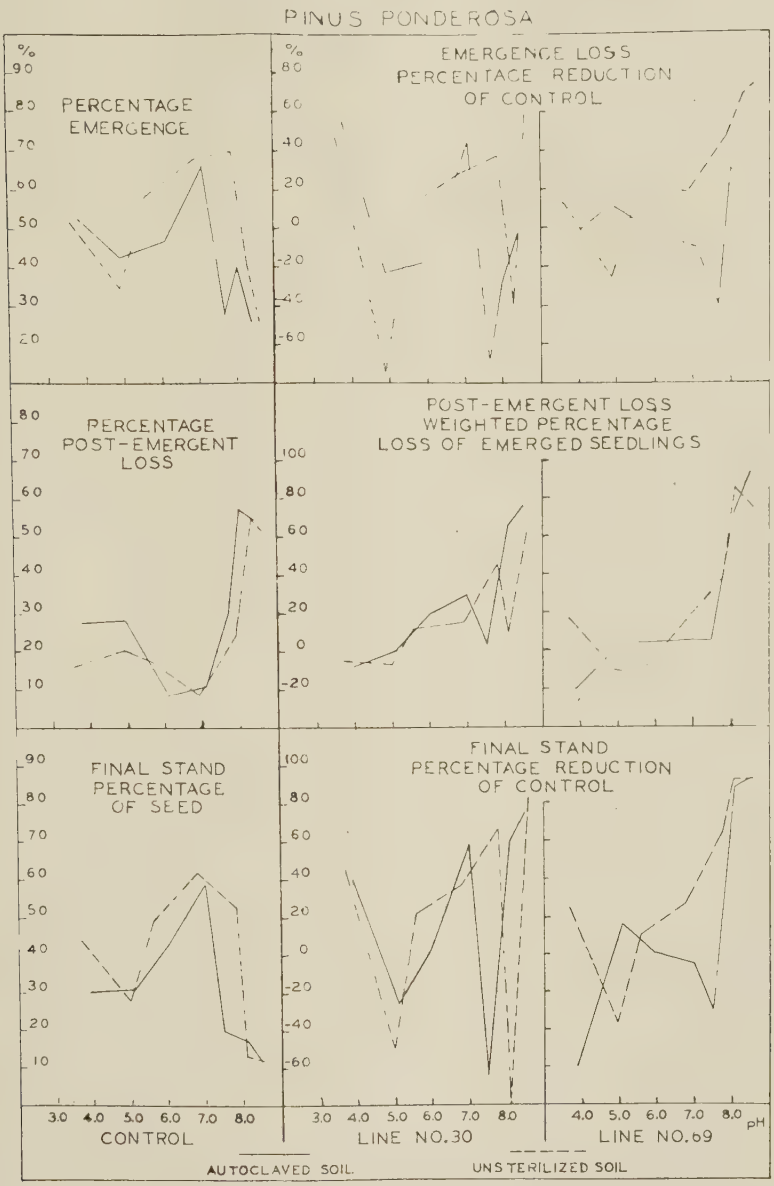

FIG. 4. Relation of pH to the growth and damping-off of *Pinus ponderosa* in soil cultures inoculated with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results for inoculated series expressed in terms of corresponding control data.

at room temperature, 50 seeds each of *Pinus resinosa, Pinus ponderosa,* and *Pinus sylvestris* were planted in each pot, and all series were placed in the greenhouse.

Emergence and post-emergence losses were tabulated during 5 months. At the termination of the experiment a survival count was made and the final pH of the various soils was measured. To evaluate the effects of the inocu-

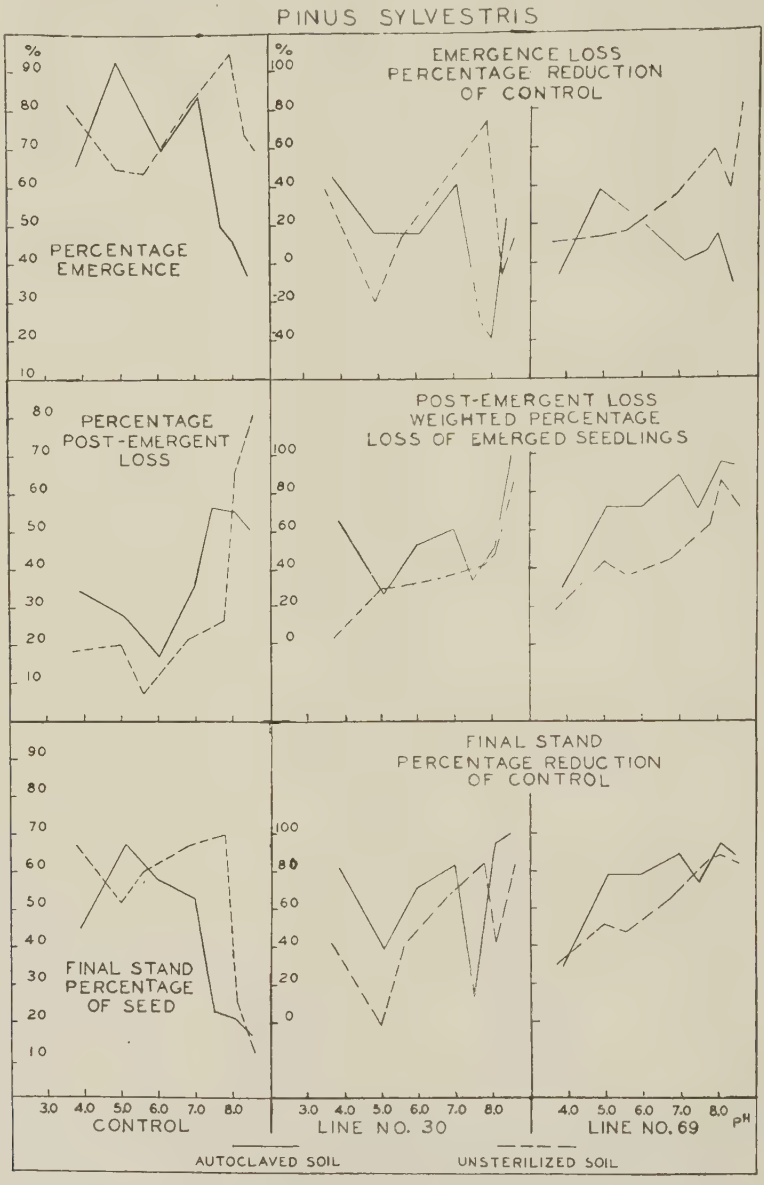

FIG. 5. Relation of pH to the growth and damping-off of *Pinus sylvestris* in soil cultures inoculated with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results for inoculated series are expressed in terms of corresponding control data.

lations alone at the various pH levels, apart from those losses occurring as a result of the growth of the hosts in some instances in a physiologically unsuitable environment, seedling losses in inoculated pots were calculated in

terms of corresponding control data. The weighted results are given in figures 3, 4, and 5, which also include the original control results for purposes of comparison. Emergence data are plotted against initial pH values, and those for post-emergence loss and final stand against the average reactions that prevailed during the experiment.

The differences in pH, or their indirect effect, exerted a pronounced influence upon the relative emergence of the hosts, and also affected their ability to survive. Attempts to isolate organisms from seedlings that died in control pots showed that fungi could not be isolated from approximately half the seedlings, and that from infected seedlings, Fusarium isolations were no more numerous than other contaminants.

TABLE 1.—*Final concentrations in parts per million of some constituents determined by dilute-acid extracts of soil samples adjusted and maintained in a pH range. A blank test indicates the substance is low beyond the sensitivity of the test reaction. The values for pH are average for the duration of the experiment*

Ions	pH values for autoclaved soils						
	3.9	5.1	6.0	7.0	7.5	8.1	8.5
Ca	40	110	115	160	150	165	175
K	0	0	0	0	0	0	0
NO_3	1–2	10	8	5	5–8	18	20
NO_2	0	0	0	tr	1	tr	tr
NH_3	5	6	2–3	0	tr	2	1
Mg	1–2	5	7–10	8	10	15–20	8
P	2	4	5–6	10	45	25	30
SO_4	30	120	10	0	0	0	0
	pH values for unsterilized soils						
	3.7	5.0	5.6	6.8	7.8	8.1	8.6
Ca	40	100	90	165	175	250	200
K	0	0	0	0	0	0	0
NO_3	5–10	15	5	2	4–5	10–14	60
NO_2	0	0	0	0	1	1	tr
NH_3	3	2	tr	0	1–2	1	1
Mg	1–2	3	8–10	12–16	10–12	12–14	12
P	2–3	5	5	8–10	15–16	45	55
SO_4	50	100	0	tr	0	0	0

At the several levels of acidity, there were pronounced differences in host-response with respect to emergence, survival, and damping-off to two Fusarium lines, both in autoclaved and unsterilized soils. However, there was no indication whether the effects were due to reaction differences directly, or to the indirect results of the chemical changes induced by the treatments of the soil samples. Analyses made at the termination of the experiments upon the uninoculated soil samples, according to the methods of Spurway (34), showed significant differences in the availability of certain constituents at the different levels of reaction, and between autoclaved and unheated samples. The results of these analyses are given in table 1. Certain of these differences could have been anticipated from the manner of soil treatment. Thus the increase in Ca availability with the rise in alkalinity

and of the SO_4 ion in the acid samples are understandable. The latter, however, gave a higher test at pH 5 than at the more acid levels to which it had been added in greater amounts. In view of similar variability of the other

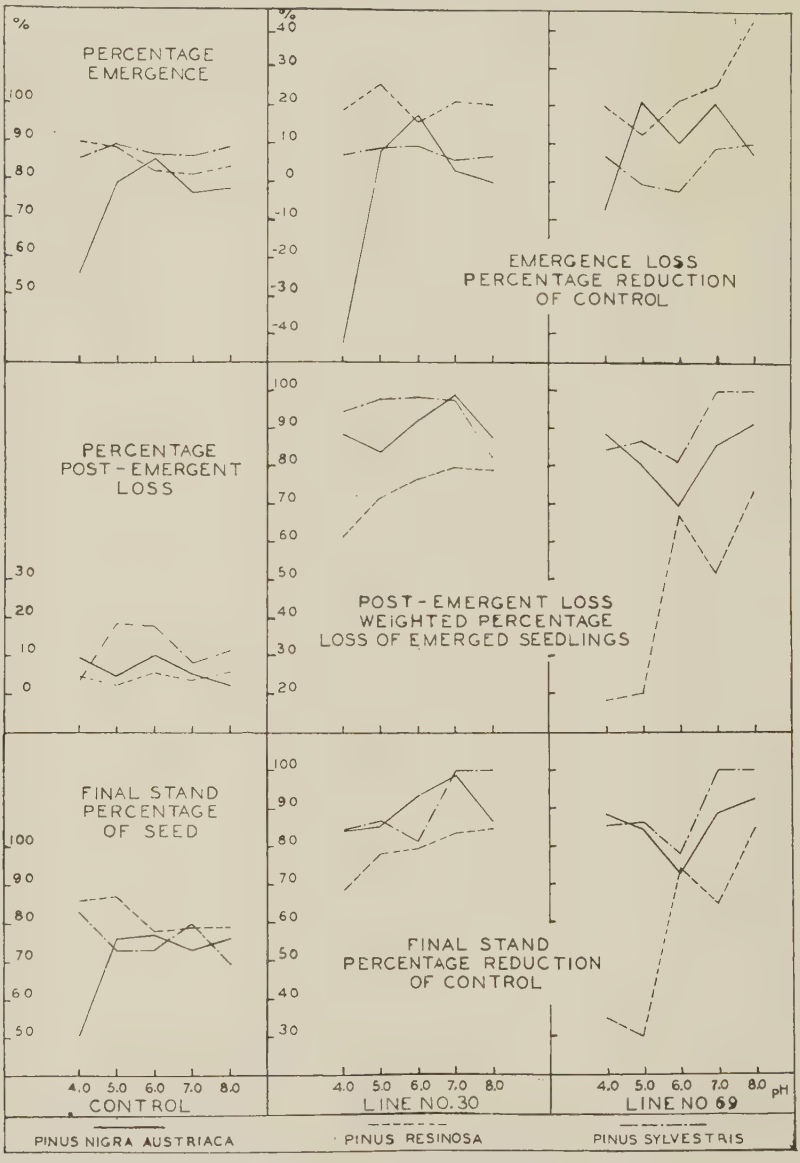

FIG. 6. Relation of pH to the growth and damping-off of three conifers in quartz-sand cultures inoculated with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results for inoculated series are expressed in terms of corresponding control data.

constituents, further experiments were necessary, in which the factors of reaction and nutrition could be independently controlled.

In order to establish the relationship of acidity alone to the development

and damping-off of conifers, inoculation tests were made in quartz-sand (Ottowa) substrates, watered by flooding daily with the basic nutrient solution adjusted in pH at one-unit intervals from 4 to 8 inclusive. The experiments were in duplicate on three hosts inoculated with two fungi, *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Fifty seeds of *Pinus resinosa* and 50 of *Pinus sylvestris* were sown separately in four-inch pots, and the same number of seeds of *Pinus nigra austriaca* in five-inch pots, all of which had been inoculated five days previously with rice cultures of the fungi. Control series received sterile rice mush. Emergence and losses were recorded for two months. The results, recalculated according to the method described, are summarized in figure 6.

Corresponding studies were made upon the effects of acidity variations

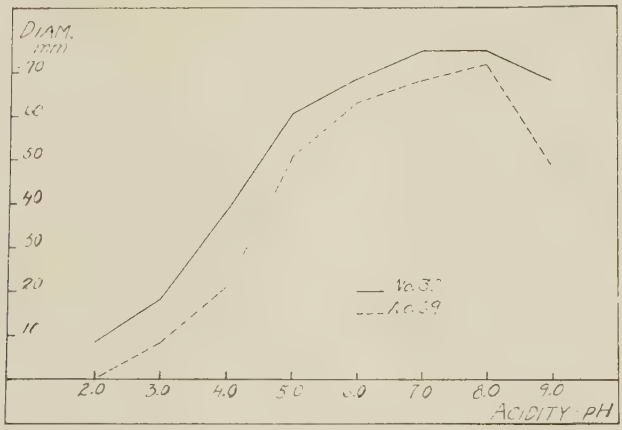

FIG. 7. Effect of acidity on the growth of *Fusarium oxysporum* (No. 30) and *F. avenaceum* (No. 69) on potato-dextrose agar.

upon the growth of the two Fusaria in Petri plates on potato-dextrose agar. The pH varied by one unit from 2.0 to 9.0, each treatment replicated five times. After incubation for six days at 28° C., two diameters at right angles were measured for each culture and averaged (Fig. 7).

NUTRITION

The concentration of the nutrient solution is an important factor in the growth and tolerance to environmental conditions of coniferous seedlings (8, 24, 25, 26, 41). A deficiency in any of the essential nutrients or an unbalanced ratio, as may well be induced by prevalent acidity conditions (13, 22, 28), influences the entire metabolic process, and as a consequence, may decrease the resistance of the seedlings to physiological and infectious diseases. Similarly, Fusarium species, as well as other soil organisms that may be potential damping-off agents, have specific nutritional requirements (17, 21, 27, 42), and the effects of alterations in concentration of nutrients, with changes in acidity, may conceivably be the underlying or a contributing factor to the various levels of pathogenicity of these organisms.

Nutritional aspects of the growth and damping-off of *Pinus resinosa* were studied in sand cultures, with excesses and deficiencies of calcium, phosphorus, nitrogen, magnesium, potassium, and sulphur in the standard nutrient solution. Since the original nutrient solution contained certain salts supplying two of these elements, in order to eliminate either one of them it was necessary to replace an equivalent concentration of the remaining constituent of the compound by sodium-salt or metallic chloride, depending upon whether it appeared as an anion or cation. An excess of any element was attained by trebling its molar concentration by the addition to the basic

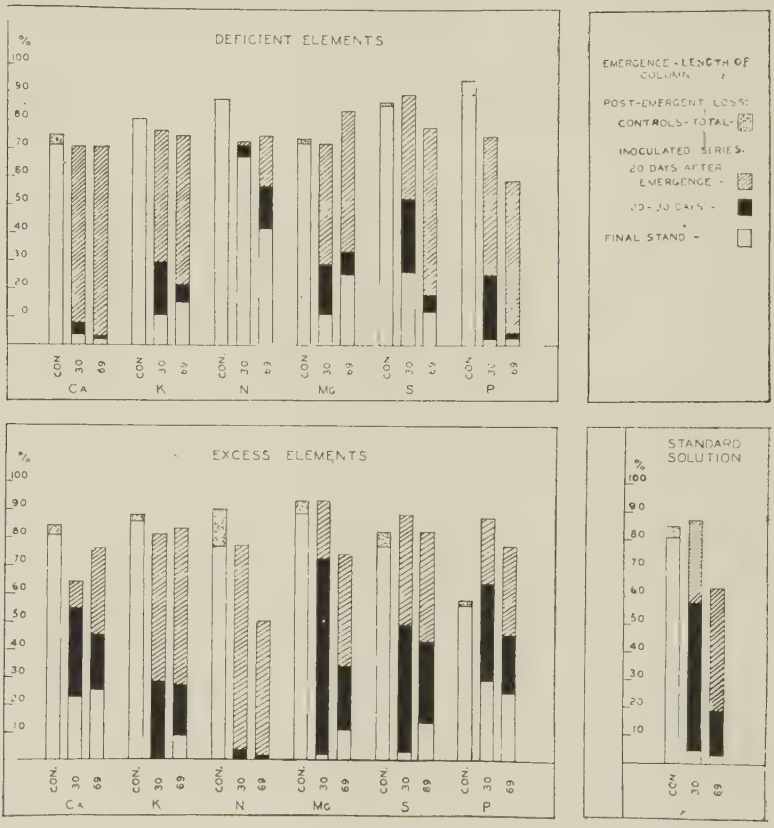

FIG. 8. Effects of deficiencies and excesses of various elements on emergence and damping-off of *Pinus resinosa* inoculated in quartz-sand cultures with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results are averages of duplicate trials.

solution of twice the original concentration of the particular ion as chloride or sodium-salt. In the case of phosphorus, additional treatment was necessary. Since originally this element was supplied in a glycerophosphate salt, used also as a source of carbon for the fungi, the removal of this ion from the solution necessitated the addition of an equivalent concentration of some carbon source, in this case dextrose. Conversely, an excess of phosphorus was attained by the use of Na_2HPO_4, in order not to increase the basic carbon content of the solutions. All solutions before use were adjusted to a final pH of 6.

TABLE 2.—*Results of the growth of Pinus resinosa seedlings in quartz-sand cultures deficient in certain elements. Duration of experiment 30 days following emergence. Daily mean temperature range 68–75° F.*

Element	No. of seedlings	Average length in millimeters		Average weight in milligrams		Ratio of green to dry weight
		Roots	Hypocotyls	Green	Dry	
Ca	23	13.7	43.9	43.4	4.6	9.4
K	21	11.2	39.3	33.9	5.5	6.2
N	30	21.5	42.4	45.6	7.1	6.4
Mg	11	10.9	35.3	43.5	5.3	8.2
S	30	12.6	39.9	44.0	4.7	9.4
P	27	12.7	43.3	44.6	3.9	11.4
Full	30	13.4	43.7	44.0	6.0	7.3

The inoculation procedure was modified to prevent any great change in the concentrations of the various nutrients which might result from the addition of rice to the substrate. *Fusarium oxysporum* and *F. avenaceum* were grown on 50 cc. of an aqueous extract of boiled rice, to which had been added 0.5 per cent dextrose. After incubation for three weeks at 28° C., the mycelial mats were removed, thoroughly washed with distilled water, and then ground with sterile sand in a mortar. This was distributed as inoculum, at the approximate rate of one-half mat per pot, into four-inch pots of autoclaved sand, which were subsequently saturated with the various nutrient solutions. The pot cultures were incubated for five days at room temperature and 50 seeds of *Pinus resinosa* were then sown in each. Duplicate series for both fungi and controls were maintained for 30 days following general emergence of the seedlings, the pots being watered daily by flooding with the corresponding solutions.

The results are given in figure 8. At the termination of the experiment, the seedlings were removed from the substrate and certain growth characteristics measured. These have been summarized in tables 2 and 3.

The responses of *Fusarium oxysporum* to nutritional conditions were tested in 5 replicates on agar substrates (1.5 per cent) in Petri dishes, pre-

TABLE 3.—*Results of the growth of Pinus resinosa seedlings in quartz-sand cultures with excesses of certain elements. Duration of experiment 30 days following emergence. Daily mean temperature range 68–75° F.*

Element	No. of seedlings	Average length in millimeters		Average weight in milligrams		Ratio of green to dry weight
		Roots	Hypocotyls	Green	Dry	
Ca	30	20.5	38.4	41.2	5.7	7.2
K	30	19.5	39.6	43.0	5.7	7.5
N	22	18.6	38.8	42.8	5.9	7.3
Mg	36	20.6	38.9	44.7	5.5	8.1
S	35	19.2	39.1	41.8	5.6	7.5
P	24	18.2	37.7	43.5	5.8	7.5
Full	32	20.1	39.9	46.0	5.6	8.2

pared from solutions containing corresponding excesses and deficiencies of elements, and adjusted to yield pH 6 after sterilization. The average diameter of each colony was determined after incubation for 6 days at 28° C. The results, given in table 4, indicate significant differences in the growth of the fungus under the several conditions. Removal of a single element in each case reduced the growth in comparison to that on the standard solution.

TABLE 4.—*Results of the growth of Fusarium oxysporum in synthetic-nutrient agar containing deficiencies and excesses of certain elements*

Element	Average diameters of colonies in mm.	
	Deficiency	Excess
Ca	74.7	84.5
K	69.0	81.4
N	65.7	86.4
Mg	72.6	80.1
S	69.4	82.6
P	67.6	78.5
Full	82.4	

DISCUSSION

Inoculations in sand cultures, adjusted over a pH range, with two species of *Fusarium* upon three coniferous hosts, revealed, on the average, a direct correlation between reaction and damping-off. Although variance was evident in the reactions of the hosts among themselves and between fungus treatments, a definite trend nevertheless was indicated for losses to become greater with decreasing acidity. The differences in parasitism at the various pH levels were most likely dependent upon the direct effect of the reaction of the media on the growth of the pathogens. With the single exception of the reduction in emergence of *Pinus nigra austriaca* on the most acid medium, different levels of reaction apparently had no significant effects upon the hosts, measured in terms of emergence and survival. In contrast, however, both fungi were directly affected by the changes in acidity, and their relative growth at the various levels was directly correlated with the losses induced. Jackson (16) also has interpreted the lower losses under more acid conditions with inoculations of *Pythium* and *Rhizoctonia* on conifers in sand and liquid cultures, in terms of a direct effect of the reaction on the growth of the parasites.

The results of soil tests of reaction differences indicated a far greater variability for damping-off as well as for emergence and survival of hosts in uninoculated controls. In both autoclaved and unsterilized soils, losses were generally negligible or moderate at the extreme acid end of the range. Maxima were reached approaching neutrality and the losses dropped sharply in most cases at pH 7.5 and 8.0, to be followed later by severe or complete losses at the most alkaline values. Roth (29), reporting results from a study of the effect of pH on the damping-off of Norway spruce (*Picea excelsa* Link) grown under somewhat similar soil conditions, found losses caused by *Cor-*

ticium vagum and *Pythium deBaryanum* definitely increased from pH 3.8 to maxima between 6.5 and 7.0 and then decreased at pH 8.0. These facts agree to some extent with the present results, although they were not extended so far with regard to more alkaline substrates.

The variance between the results derived from sand- and soil-cultures undoubtedly resulted from the additional chemical variability in the latter. Deficiencies and excesses of certain elements which varied in the soil cultures, tested under controlled conditions of reaction in sand-culture, were associated with losses from damping-off. It was frequently possible to correlate the magnitude of the attack with the simultaneous effects of these nutritional conditions upon the host and pathogen. Varying nitrogen gave the most pronounced differences in this respect. Where this element was deficient the growth of the fungus was least, whereas the host had optimum root development as well as a relatively low degree of succulence, expressed as ratio of green to dry weight. There was little loss from damping-off. An excess of nitrogen, although mildly affecting the host by a slight reduction of root length, unquestionably stimulated fungus growth sufficiently to account for the rapidity and severity of its subsequent attack. Hartley (10) has likewise expressed the belief, based on general nursery experience, that conifers grown on a soil rich in nitrogen are especially susceptible to damping-off.

Illick and Augenbaugh (15), in studies on *Pinus rigida,* stated that an excess of lime in the soil creates conditions which are most favorable for the activities of damping-off fungi, although no mention is made of any direct effect of excess lime on the seedlings. In the present experiments, a deficiency of calcium, inducing a high degree of succulence of the seedling, combined with the best fungus growth of the entire deficiency series, resulted in early and heavy attack. On the other hand, an excess of calcium yielded a hardened seedling with good root development, by virtue of which the host apparently was able to withstand rather good growth of the fungus. This suggests that the high mortality associated with alkaline calcareous soils is due to the reaction directly rather than to the high calcium content. In fact, Albrecht and Jenny (2), in studies of the damping-off of soy bean seedlings, concluded that within a wide range of pH (3.8–8.0), heavy damping-off occurred only when the calcium supply was low, but none took place when the supply of calcium was high. Chapman (6) indicated that pH and soluble-calcium content may independently influence survival of shortleaf pine (*Pinus echinata* Mill.) ; the latter factor, however, restricting survival with concentrations of 500 parts per million or higher. Howell (14) observed that calcium is not deleterious to seedlings of *Pinus ponderosa,* and suggested that the fact that the ion is not assimilated by the plant in alkaline soils may be important. In the soil experiment of the present investigations, the calcium content was increased simultaneously with the alkalinity of the medium, and the higher losses that resulted may have been due to the masking of the beneficial effect of calcium, present in less than toxic concentrations, by the alkaline environment.

The concentration of phosphorus likewise markedly affected the growth of the host as well as the extent to which it damped-off. Under a deficiency of this element, the seedlings were the most succulent of the entire lot and were extremely susceptible to attack in spite of the fact that the fungus grew rather poorly. With an excess of phosphorus, the seedlings became moderately hard and the fungus growth was poorest of the entire excess series, both factors combining to yield a relatively lower level of attack. Availability of this element appeared to be a factor related to the reduction of losses in the soil experiments on the alkaline side of neutrality. These low points generally occurred at pH 7.5 in the autoclaved soils and at 8.1 in the unsterilized lots. Both points coincided with a high content of available phosphorus. The availability of this element has been associated with buffering of plant cytoplasm and cell sap (6), its reduction in the substrate being associated with a lower degree of buffer capacity for alkali. Addition of superphosphate to an acid nursery soil in which seedlings were developing, somewhat increased the ability of the plants to withstand a wide range of environmental conditions (14).

Damping-off of the seedlings, under the influence of variations in the concentrations of the other elements tested, was less consistent than were the effects of the conditions upon the hosts. In general the reduction of losses where the test elements were deficient, as compared with the control, appeared to be related to the corresponding reduction in growth of the fungi. In those media in which all the elements were present in their basal concentrations, the growth of the colonies appeared little influenced by specific excesses, and the losses were comparable with those in the standard solution. Young and Bennett (42) have shown that the proper balance of the inorganic constituents in the solution is essential for the maximum growth of *Fusarium oxysporum* and other fungi. In the present experiments, this balance was more affected by the removal of an element from the nutrient solution than by an increase in its concentration.

The chemical factors were connected with steam sterilization of soil. Heating the soil produces pronounced chemical and biological changes (10, 23, 30, 32), which must be considered in evaluating the treatment to control damping-off. Lindfors (19) concluded that steam-sterilization of soil created conditions favorable for reinfection by Fusarium. Ten Houten (35) found a similar increase in attack with *Rhizoctonia solani* on sterilized soil, and attributed the higher damage to the removal by the heating process of organisms antagonistic to the pathogen. A similar view was taken by Hartley (10). Scheffer (30) observed that a heat treatment of soil was not detrimental to subsequent growth of several conifers; in fact, plants grown in the heated soil developed somewhat better roots and crowns. However, as indicated by the results of the present experiments as well as other observations (23, 32), steam sterilization may change the reaction of a soil as well as its basic nutritional equilibrium. Some of these factors may operate independently, or in combination, upon host and pathogen alike to affect directly the

amount of disease. Thus the effectiveness of the treatment depends upon whether the conditions favor or oppose the relationship. The present results have indicated that the treatment increased subsequent losses as often as it reduced them, with no apparent consistency, over the entire range of soil samples. Similar conditions in the field undoubtedly account for the variability and contradiction of the reports on the usefulness of this method.

SUMMARY

In seedlings of *Pinus resinosa* grown in quartz-sand culture and inoculated with Fusaria and other fungus lines, losses due to more virulent fungi appeared relatively early and reached their maximum levels within approximately three weeks following emergence, whereas those due to less virulent lines were delayed, although generally reaching maximum levels before thirty days. Losses from *Sclerotium bataticola,* a comparison line, were low for the first six weeks, but increased thereafter and attained a high level before seventy-five days.

Tests with seedlings of various ages, inoculated with *Fusarium oxysporum* under identical conditions, demonstrated that resistance to invasion increased directly with increasing age of *Pinus resinosa.*

The relation of acidity to damping-off was tested in cultures of a single soil the pH of which was varied artificially. In samples, subdivided to include autoclaved and unsterilized lots, emergence reductions of three coniferous species due to inoculations with *Fusarium oxysporum* and *F. avenaceum* were generally low or moderate at pH values in the acid range and high above pH 8. In some cases emergence in inoculated pots was greater than in corresponding controls at pH 7.5 in autoclaved samples and at pH 4.9 and 8.3 in unsterilized soils. Post-emergence damping-off was low in more acid samples, reached a maximum on the acid side of neutrality, fell off sharply in the autoclaved soil at pH 7.5 and in the unsterilized soil at pH 8.1, and then became uniformly high in the most alkaline substrates. Differences were not consistent at the various levels of acidity from autoclaved and unsterilized soil samples. The chemical variability in the soil samples, which resulted from the induction of reaction differences and from steam-sterilization is discussed.

In quartz-sand cultures of varying pH, losses of three conifers inoculated with the same fungi were generally high at all levels of acidity, although there was some tendency for increased damping-off with increasing alkalinity.

Tests in quartz-sand cultures on the effects of variations in the concentration of certain constituents of the nutrient solution, under uniform conditions of reaction, demonstrated that the influence of nutritional factors on damping-off could be correlated with their simultaneous effects upon the host and pathogen. The magnitude of the loss was associated with the growth of the pathogen and the degree of susceptibility of the host, measured in terms of its succulence. A deficiency of nitrogen gave relatively negligible

losses, whereas an excess caused early and heavy attack. Deficiencies of calcium and phosphorus resulted in high damping-off losses, which were considerably reduced when these elements were present as excesses. When potassium, sulphur, and magnesium were deficient, final stands were somewhat higher than under excesses of these elements, the latter resembling controls grown and inoculated in standard solutions.

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STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. III. RELATION OF TEMPERATURE AND SUNLIGHT TO THE PATHOGENICITY OF FUSARIUM[1]

HOWARD TINT[2]

(Accepted for publication January 15, 1945)

Comparatively little attention has been given to the relation of temperature and sunlight to damping-off as predetermining conditions affecting the levels of pathogenicity in the host-pathogen relationship. The close interrelationship in the field between temperature, sunlight, and moisture factors has made it difficult to interpret observations in terms of any one factor. Only when these conditions are controlled and varied independently in the laboratory, can their relations to the disease be interpreted.

GENERAL MATERIALS AND METHODS

The selection of hosts and damping-off fungi was based upon results from previous experiments (22, 23). In the reports on these tests, sources of seeds and of the Fusarium lines were described in detail, as well as standard methods of seed sterilization, selection of monosporous cultures of virulent strains of fungi, and preparation of inoculum and glycerophosphate nutrient solutions for the hosts and fungi. Damping-off losses were considered in two categories, emergence loss and post-emergence damping-off. Where experimental procedure required occasional departures from the standard methods of these early experiments, the alternative treatments are described.

All seedlings were grown in a low-humidity greenhouse, thermostatically adjusted to maintain a daily mean temperature range of 68–75° F., in those seasons of the year when such control was possible.

Determinations of pH were made potentiometrically with quinhydrone and saturated calomel half-cells, and all solutions were adjusted in acidity with 0.1 N HCl and 0.1 N NaOH.

TEMPERATURE

Various writers have considered effects of soil temperature on seed germination and early growth of forest-tree seedlings (1, 6, 14). Excessively high temperatures following high insolation exert lethal effects upon coniferous seedlings (2, 9, 25). Less attention has been given to the effects of moderate temperatures in predisposing seedlings to damping-off, although it has been generally recognized that temperature variations influence the activities of damping-off and other soil organisms (17, 21).

Fusarium species in general are adapted to a wide range of temperature, but usually develop best at relatively high temperatures, with optima in the

[1] A portion of a thesis submitted to the faculty of the Graduate School of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

range 20–35° C. (12, 16, 26). Wollenweber (27) made the generalization that root-invading Fusaria are warm-soil organisms. Other investigators (11) saw a correlation between high-temperature Fusarium wilts and the vegetative development of the parasites, and attributed the influence of temperature to its direct effect upon the fungus. Gifford (4) and Hartley (10) observed parallelism between high temperatures and damping-off of conifers, and Roth (18) demonstrated that damping-off fungi function actively between 11° and 30° C. His two species of *Fusarium* caused appreciable damage to *Picea excelsa* under the protracted influence of temperatures between 24° and 33° C.

The influence of temperature on the development of the disease, and upon the host and pathogen separately, was studied in the greenhouse in temperature chambers described by the writer (24). Air temperatures fluctuated about means at five different levels in a manner resembling diurnal fluctuation in nature. The actual mean temperatures and their standard deviations were as follows: Chamber I, 31.5 ± 1.3° C.; II, 24.6 ± 1.6° C.; III, 20.9 ± 1.4° C.; IV, 16.4 ± 1.5° C.; and V, 10.4 ± 1.6° C. The standard error of any mean was 0.3° C. These values were independent of the greenhouse temperatures.

The first inoculation tests (Series 1) were upon *Pinus resinosa* in quartz-sand cultures. Two four-inch pots, inoculated with cultures of *Fusarium oxysporum,* and two control pots, treated with sterile rice-mush, were in each chamber. Infested pots were incubated for 5 days at room temperature, after which 50 surface-sterilized seeds of the host were sown in each and covered with sterile sand. The pots were then placed in the apparatus and kept uniformly moist by frequent watering with the standard nutrient solution adjusted to pH 6. Data recorded for inoculated and control pots are in tables 1 and 2.

In table 1 are listed the cumulative percentages of seedlings emerging after various periods, up to 125 days, after placement of pots in the chambers. Since emergence had not been completed by 125 days in Chambers IV and V, it was necessary to determine the number of viable seeds remaining in these pots, in order to distinguish between lack of emergence due to inoculation and that due to physiological effects upon the seeds of the temperature alone. The sand in all the pots was sifted through a screen and all unopened seeds recovered were again surface-sterilized and planted in sterile agar in Petri dishes. The plates were incubated at room temperature for 15 days, and the additional germination was used to correct the percentage emergence of the temperature treatments. The corrected emergence percentages are in table 2, which includes the percentage reduction in emergence due to the inoculation, based upon the corresponding controls as standards, and also the total post-emergence losses.

The optimum temperature range for the emergence of the host was 16–20° C., higher values reducing germination, while lower temperatures slowed down its rate. Reduction in emergence due to the effects of the inoculations

TABLE 1.—*Emergence percentages of seedlings of Pinus resinosa grown in control and inoculated quartz-sand cultures at various temperatures. Data are averages of duplicate trials*

Culture	Temperature, degree C.	Emergence percentages from 9 to 125 days after planting of seeds									Total emergence (per cent)
		9	12	14	16	18	40	75	100	125	
Control	31.5	6	13	17	21	24	.....	.....	.....	.....	24
	24.6	5	27	32	35	.....	.....	.....	.....	.....	35
	20.9	1	39	57	65	73	75	75	76	.....	76
	16.4	.....	.....	.....	2	5	24	44	56	70	70
	10.4	.....	.....	.....	.....	.....	3	7	9	12	12
F. oxysporum	31.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
	24.6	4	9	11	.....	.....	.....	.....	.....	.....	11
	20.9	4	13	16	24	27	29	31	.....	.....	31
	16.4	.....	.....	.....	4	6	27	38	47	52	52
	10.4	.....	.....	.....	.....	.....	3	7	12	14	14

was directly correlated with temperature, maximum losses occurring in the warmest chamber and minimum in the coldest. A similar correlation with temperature was evident for post-emergence losses.

Some seedlings failed to survive in the control pots, particularly at the warmer levels. *Fusarium oxysporum* and other fungi and bacteria were isolated from such dead seedlings. Nutrient-saturated cultures maintained for a long time in closed chambers at high temperatures provided a highly suitable environment for the development of a fungal flora. Whether failures to survive in such controls were due to lethal effects of temperature upon the host, to the saprophytic organisms entering upon death of the host, or to direct pathogenic attack by these organisms upon the weakened host, could not be determined. Infection of controls with *F. oxysporum* could be directly attributed to contamination as a result of the surface-watering of inoculated and control pots in close proximity. By exercising special care in handling and watering pots while in the chambers, losses in control pots in later tests were reduced to comparatively low levels.

TABLE 2.—*Corrected emergence percentages and post-emergence losses in seedlings of Pinus resinosa grown in temperature chambers*

Culture	Temperature, degrees C.	Corrected percentage emergence	Emergence as percentage reduction of control	Percentage post-emergence loss
Control	31.5	24	.....	61.1
	24.6	35	.....	27.9
	20.9	76	.....	11.9
	16.4	77	.....	1.3
	10.4	74	.....	0.0
F. oxysporum	31.5	0	100.0	.....
	24.6	11	68.6	100.0
	20.9	31	59.2	89.6
	16.4	54	29.8	8.7
	10.4	70	5.4	0.0

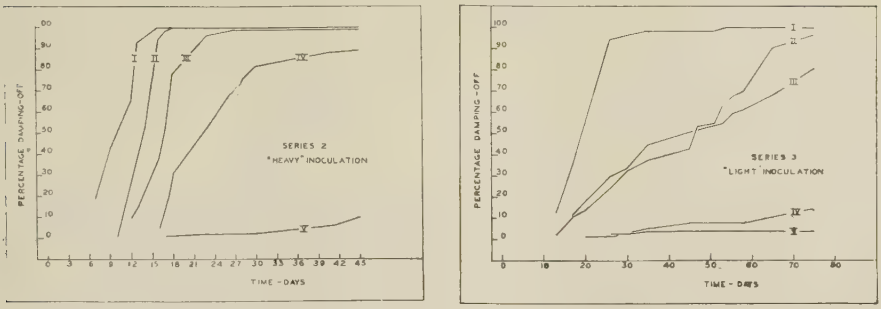

FIG. 1. Effects of temperature on damping-off in seedlings of *Pinus resinosa* in quartz-sand cultures with "heavy" inoculations and with "light" inoculations of *Fusarium oxysporum*.

Since seeds germinated at different rates in the various chambers, the emerged seedlings were exposed for different lengths of time to the fungus. Therefore, the degrees of post-emergence attack at the several temperature levels were not wholly comparable. In order to allow uniform exposure of the seedlings with respect to time, two additional experiments were made (Series 2 and 3). Seeds of *Pinus resinosa* were germinated under sterile conditions and the seedlings transplanted, when their radicles were 1–3 cm. long, to inoculated and control pots which were then placed in the chambers. In Series 2, the standard moderately heavy inoculation procedure was employed. In Series 3, this method was modified as follows: the contents of a flask of rice inoculum were shaken with 50 cc. of distilled water, and the suspension was then used to inoculate two pots of sand. Controls were similarly prepared with a suspension from sterile steamed rice. Thus, inoculation was relatively light in Series 3. Damping-off losses were recorded periodically during 45 days in Series 2 and for 75 days in Series 3. The results are in figure 1.

Upon completion of these tests, the seedlings were removed from the control pots and certain growth responses to the different temperatures were

TABLE 3.—*Effects of temperature upon the growth of seedlings of Pinus resinosa in quartz-sand cultures*

Series and duration	Temperature, degrees C.	Number of seedlings measured	Average length in millimeters		Average weight in milligrams		Ratio green to dry weight	Survival in percentage of original stand
			Roots	Hypocotyls	Green	Dry		
Series 2, 45 days	31.5	19	14.7	40.7	50.5	3.8	14.5	78.0
	24.6	30	15.8	40.9	45.8	5.0	9.2	100.0
	20.9	30	19.7	42.6	43.8	5.3	8.3	98.0
	16.4	30	17.0	39.4	40.7	5.4	7.5	100.0
	10.4	30	16.3	36.9	38.1	4.9	7.8	100.0
Series 3, 75 days	31.5	12	18.0	53.0	57.5	5.0	11.5	56.0
	24.6	20	19.1	47.8	45.9	6.7	6.8	84.0
	20.9	25	19.6	49.6	41.9	8.1	5.2	92.0
	16.4	27	18.3	49.1	41.2	7.3	5.6	100.0
	10.4	30	19.2	44.8	40.2	7.4	5.4	100.0

measured (Table 3). Final stands were expressed as percentages of the original plantings. Representative sets of seedlings from both series were photographed (Fig. 2).

Post-emergence losses in both series were correlated with temperature, as was emergence reduction in Series 1. Most rapid and greatest losses

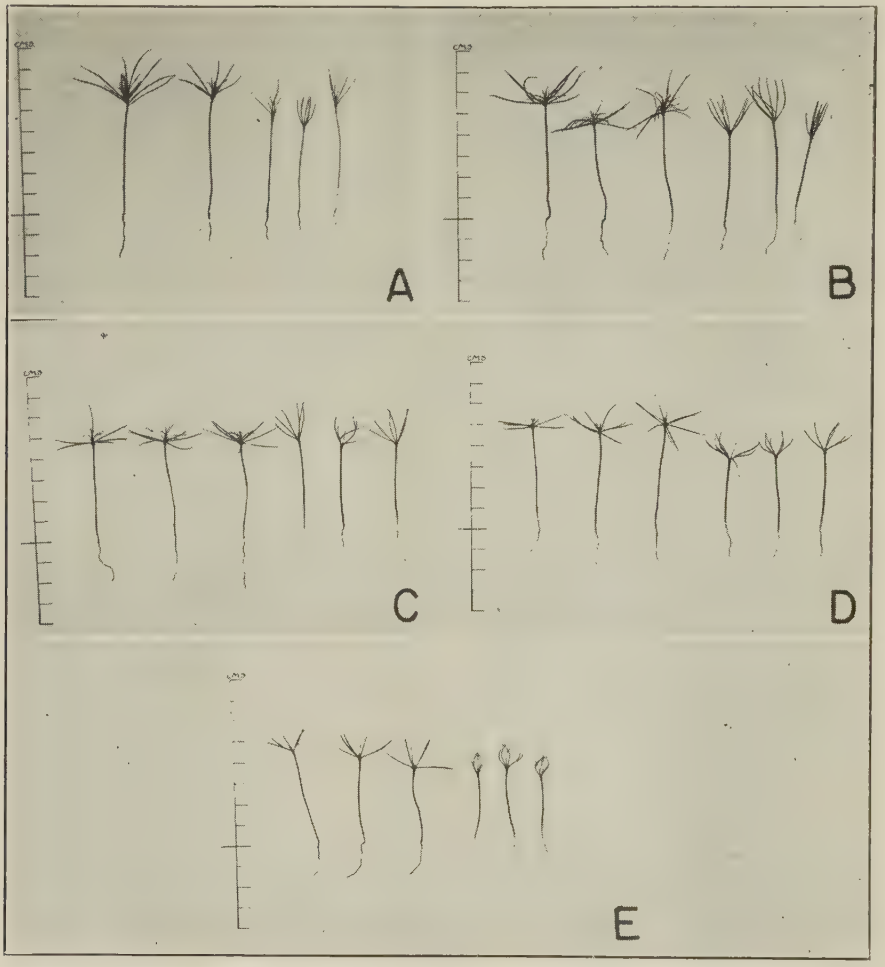

FIG. 2. Representative seedlings of *Pinus resinosa* grown in temperature chambers: A. Chamber I, 31.5° C.; B. Chamber II, 24.6° C.; C. Chamber III, 20.9° C.; D. Chamber IV, 16.4° C.; E. Chamber V, 10.4° C. The 3 larger seedlings on the left in each set are from Series 3 (75 days duration) and those on the right from Series 2 (45 days). Scale in centimeters.

occurred in the warmest chamber; with decreasing temperatures, total losses became less and seedlings were attacked less rapidly. In the same experiment, variations in temperature affected the development of the host. The most rapid growth of tops, as expressed by elongation of hypocotyls and development of leaves, occurred in direct relation to increasing temperatures

(Table 3 and figure 2). The "warm" plants, however, had weaker stems and less pigment than the "cold" ones. The characteristic increasing succulence with rise in temperature was further reflected in the ratios of green to dry weight of the seedlings. The ratios were higher at the higher temperatures.

In both series, final stands of controls were reduced at the higher temperatures. Since some dead seedlings from these controls were infected by contaminating organisms and others were free of infection, it was not possible to ascertain whether the total reduction was due to infection by these contaminants or to lethal effects of the high temperatures. If the former was the case, then unquestionably the higher temperatures predisposed the control seedlings to invasion.

Temperature effects on the growth of *Fusarium oxysporum* were tested in Petri dishes of potato-dextrose agar, adjusted to a final pH of 6 and incu-

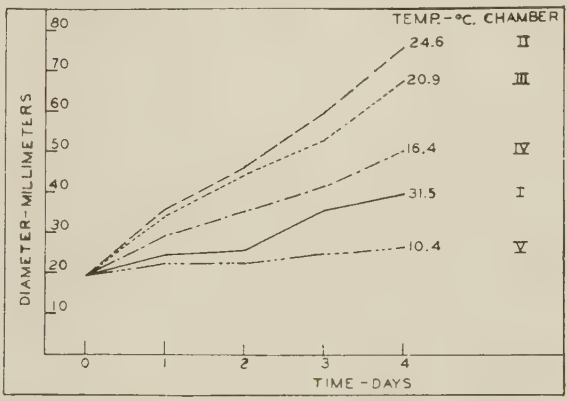

FIG. 3. Effects of several levels of temperature on the growth of *Fusarium oxysporum* on potato-dextrose agar.

bated 24 hours at room temperature before distribution to the chambers. The 5 dishes in each chamber were covered by continuously moistened cloth to equalize as far as possible the humidities at the different temperature levels. All the chambers were given normal exposure to light in order to subject the fungi to the same conditions which would prevail during the inoculation tests, although less fluctuating temperature equilibria could have been obtained in darkness. Colony diameter was measured after each 24-hour period for four days. The daily average diameters at each level of temperature are summarized in figure 3.

The fungus grew over the entire temperature range. Both in daily-increment and total-growth measurements, its maximum growth was at approximately 25° C., growth was slight at 31.5° C. and minimum development was at 10.4° C. The optimum was generally comparable with that of 25°–30° C. reported for the growth in pure culture of *Fusarium oxysporum* isolated from potato-wilt by Goss (5), although he demonstrated that the value varied with the substrate.

SUNLIGHT

The relationship of light to the damping-off problem is an indirect one. Diurnal and seasonal variations in insolation are the main sources of the temperature and moisture fluctuations that occur in the field and affect seedling development (1). Furthermore, light affects growth and survival directly (3, 7, 15, 19, 20). Smith (21) pointed out that the heavy shading of seedlings retards growth and prolongs succulence in a manner that may contribute to damping-off losses. Since nursery practice commonly employs shading in seedling beds, further knowledge is essential on the direct relation of light to damping-off.

The effects of variations in light intensity on the damping-off of coniferous seedlings were studied in the greenhouse. Slotted screens provided varying degrees of illumination (Fig. 4). These were preferred to shading

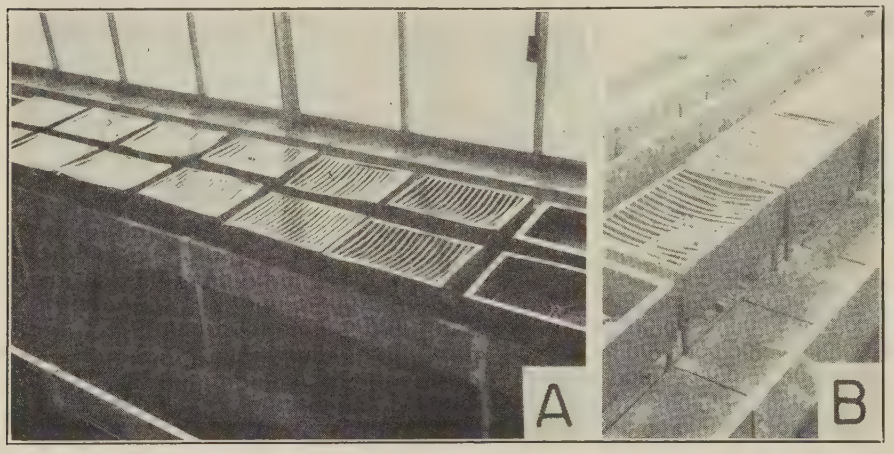

FIG. 4. A. Shading-screens providing varying degrees of radiation-intensity ranging from 0 per cent, upper left, to 100 per cent, lower right. B. View showing manner of supporting frames on legs.

cloths since lath shades are more common in nursery practice. They also provided conditions more closely resembling those under a natural canopy of vegetation, where plants are alternately exposed to high and low light intensities. The panes of the greenhouse were of ordinary clear glass, allowing most of the light to pass through.

The screens and supporting frames ($12 \times 18 \times 6$ inches) were stiff corrugated paper and were painted on all surfaces with nonreflecting black paint. Each frame was supported on legs to raise its lower edge 3 inches above the surface of the bench and permit free circulation of air. At the same time the light-intercepting area was thus sufficiently elevated over the surfaces of the pots so that light intensity, measured with a photo-electric cell (Weston) was uniform at all locations in the seedling level. The width and spacing of the openings of the screens were planned to permit, after calibration with the photometer, various percentages of the light-intensity in the open frame:

75 per cent, 50 per cent, 25 per cent, 12 per cent, 6 per cent and 0 per cent. (See Fig. .4) Each shade frame covered two 7-inch pots.

Duplicate inoculations were made in autoclaved soil (3 parts loam, 2 sand, and 2 leaf-mold) with *Fusarium oxysporum, F. vasinfectum* and *F. avenaceum,* and each pair was tested under a single frame at each level of light-intensity. Fifty surface-sterilized seeds of *Pinus resinosa* and of *Pinus sylvestris* were sown in each inoculated pot after 5-days' incubation of the fungus. Post-emergence losses of seedlings through damping-off were recorded for 30 days. The tests were carried out on the south side of the greenhouse, the apertures of the screens arranged at right angles to the path of

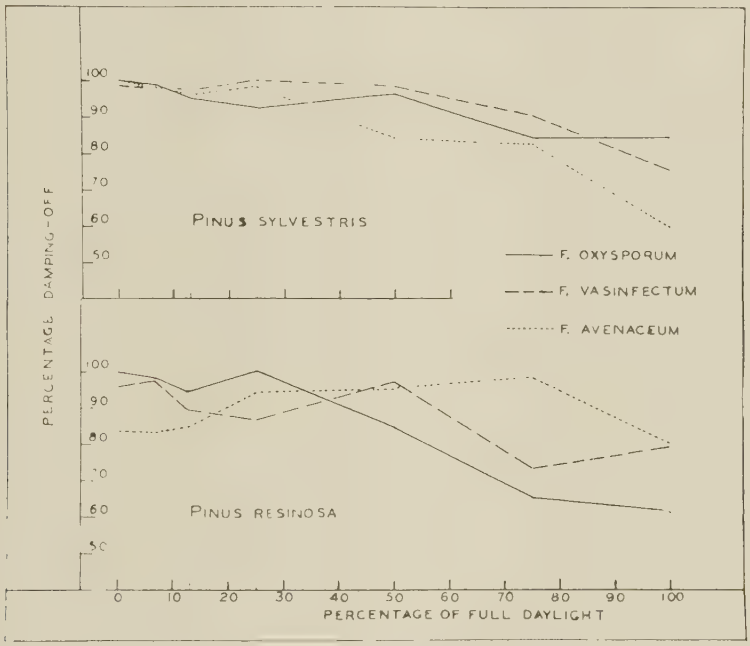

FIG. 5. Relation of light-intensity to damping-off of *Pinus resinosa* and *Pinus sylvestris* in sand cultures inoculated with *Fusarium oxysporum, F. vasinfectum,* and *F. avenaceum.* Data are based upon duplicate trials.

the sun. Damping-off losses in relation to light-intensities are represented in figure 5.

An additional set of uninoculated seedlings was tested, in duplicate pots, in the light-intensity range. The growth characteristics were measured 30 days after emergence (Table 4); and representative seedlings of both hosts grown under each light intensity are shown in figure 6. Succulence in both hosts, measured in terms of hypocotyl elongation and ratio of green to dry weight, increased with decreasing light-intensities. Root length was optimum for *Pinus resinosa* in 50 per cent and 75 per cent light and for *P. sylvestris* in 75 per cent light. The differences in root length, however, were probably an index to temperature variations due to the different degrees of insolation, although the practice of watering frequently during the day,

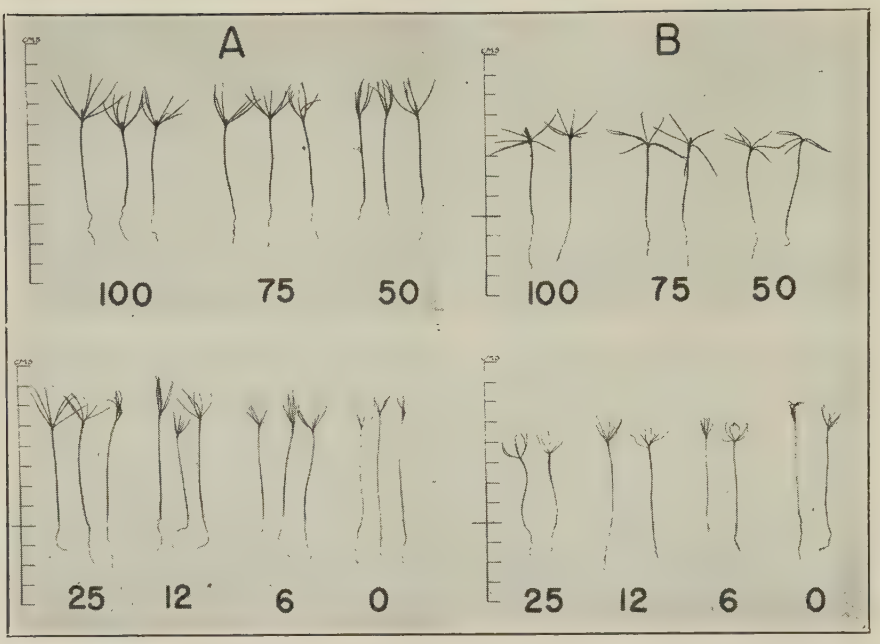

FIG. 6. Representative seedlings of *Pinus resinosa* (A) and *Pinus sylvestris* (B) grown for 30 days under different light-intensities (percentages of full light given numerically).

keeping soil-moisture content uniform in all pots, tended to keep temperatures from fluctuating too widely. Below-surface ($\frac{1}{4}$–$\frac{1}{2}$ inch) temperatures of the soils on the average varied from 18° to 23° C., and the maximum attained in any pot was 28° C. in pots exposed to full maximum solation.

The effects of similar light-intensities upon the growth of the fungi were measured. Lundegårdh (13) cited instances of the penetration of light rays

TABLE 4.—*Relation of light intensity to the growth of Pinus resinosa and Pinus sylvestris in autoclaved-soil cultures. Duration of test 30 days following emergence*

Plant	Percentage of full light	No. of seedlings measured	Average length in millimeters		Average weight in milligrams		Ratio of green to dry weight
			Roots	Hypocotyls	Green	Dry	
Pinus resinosa	0	22	17.1	50.8	30.5	2.4	12.7
	6	30	17.6	51.9	44.4	4.2	10.6
	12	30	16.1	48.1	43.0	4.7	9.2
	25	30	17.1	49.7	43.2	4.4	9.8
	50	30	19.9	46.2	43.4	5.3	8.2
	75	30	19.8	42.7	43.4	5.9	7.4
	100	30	16.9	38.2	44.0	5.8	7.6
Pinus sylvestris	0	24	17.0	45.8	35.0	2.7	13.0
	6	23	15.7	41.2	32.7	2.3	14.2
	12	22	16.0	42.0	32.5	3.0	10.8
	25	24	15.1	37.2	27.5	3.1	8.9
	50	19	13.1	36.2	21.1	3.7	8.4
	75	21	20.2	37.6	37.7	4.6	8.2
	100	22	18.2	35.9	37.5	4.9	7.7

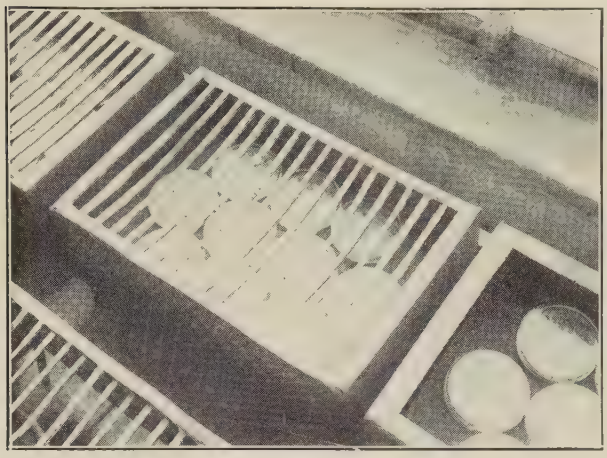

FIG. 7. Adaptation of screens for testing the relation of light intensity to the growth of fungi in Petri dishes.

below the surfaces of soils, measured in terms of the photosynthetic activities of soil algae growing at depths of several centimeters. Therefore, in the present experiments, the effects of light-intensity on the growth of the fungi probably bore some relation to the amount of disease they caused. Petri dishes of potato-dextrose agar were seeded with the fungi, five plates of each organism for each screen, and the plates were placed upon platforms at the same distance below the screens as were the soil-surfaces in the inoculation tests (Fig. 7). After exposure for 4 days, the average diameter of the colonies under the various screens was measured (Fig. 8). Growth of each fungus generally increased with reduction in light-intensity, agreeing with the observations of Harter (8), that the vegetative growth of various Fusaria was more abundant in the dark.

Under the conditions of the experiment, the effects on fungus growth of differences in temperature induced by different light-intensities could not

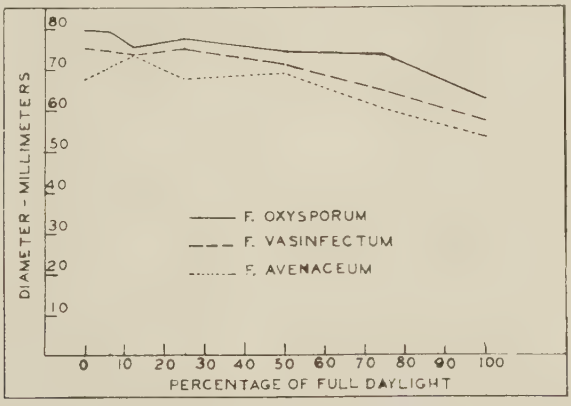

FIG. 8. Effects of light intensity on the growth of three species of *Fusarium* on potato-dextrose agar.

be wholly evaluated. Although greenhouse temperatures were maintained at 20–24° C., undoubtedly increasing insolation elevated temperatures beneath close-fitting Petri-dish covers. However, at no time were lethally high temperatures observed in any plates during the test, and periods of exposure of the plates to direct radiant heat, considered on a 24-hour basis, were relatively short.

DISCUSSION

Damping-off of *Pinus resinosa,* caused by *Fusarium oxysporum,* proved to be directly related to temperature. While high temperatures hastened the development of the host, its succulence also increased, so that its resistance to the fungus was lowered. Thus emergence and post-emergence losses were highest at temperatures beyond the optimum for fungus development. However, on the whole, the variation in losses in relation to temperature appeared to depend upon its effect simultaneously upon both host and pathogen. Increase in losses with mounting temperatures showed the same general progression with time in experiments differing in amount of inoculum, thus indicating a host response. On the other hand, the differences in the relative positions of the curves of losses between "heavy" and "light" inoculations, suggested a relation between the virulence of the fungus and the particular temperature conditions.

An important fact demonstrated by these experiments is the apparent existence of a critical temperature below which the host is able to grow and escape infection in infected soil. Roth (18) also described damage caused by Fusarium species only at high temperatures at which *Pythium* and *Rhizoctonia* were relatively harmless. In the present experiments, in both light and heavy inoculations of *Pinus resinosa* seedlings grown at an average temperature of approximately 10° C., losses were negligible. This fact showed a striking parallelism with other Fusarium wilts whose reactions to soil temperatures have been studied (11). These reports have also established minimum temperatures below which the hosts have grown fairly well and have escaped serious infections: for example, tomato wilt, 19° C.; cabbage yellows, 17° C.; flax wilt, 14° C. This phenomenon obviously is directly related to the thermophilic nature of the Fusaria generally, as has already been mentioned. The lowest temperature established in the apparatus of the present experiments, 10° C., probably did not represent the actual critical point. At an average of 16° C., the next highest temperature available, losses were low during the 75 days following a light inoculation of the fungus, and relatively high 45 days after a heavy inoculation. The critical point was probably between 10° and 16° C.

The influence of light upon damping-off was related to its effect upon the host and the pathogen. With increasing shade, both the succulence of the host and the growth of the fungi became greater, and both apparently contributed to increasing damping-off losses. These results could be distinguished from any possibly caused by secondary effects of variations in the insolation. Two factors which vary primarily in the field with the amount

of sunlight are temperature and moisture. In these experiments, moisture variation was minimized through the practice of frequent and uniform watering. The abundant moisture further served to check rising temperatures with increasing insolation. Since maximum temperature variations were recorded for non-shaded pots, if the light experiments were being mainly influenced by secondary temperature variations, then the greatest losses could be expected in the unshaded pots. However, the results indicated that the converse was true, best stands being obtained in full light. This indicated that light conditions were affecting the results independently of secondary temperature effects. In the field, however, unquestionably beneficial effects of full light are lost. Moisture control becomes more difficult with higher insolation, and the higher rate of evaporation which results causes the soil to dry out more rapidly. This, in turn, permits higher soil temperatures and increases possibilities of fungus attack.

SUMMARY

The relation of temperature variations to damping-off and to the growth of the host and pathogen was tested in control equipment.

Optimum growth of *Fusarium oxysporum* was favored by a temperature of approximately 25° C. Emergence of *Pinus resinosa* was reduced lethally by some higher temperatures in the range tested, whereas its rate was slowed down by the lower levels. In post-emergent development, rapidity of growth and succulence of *Pinus resinosa* increased with rising temperatures.

Increase in emergence losses and post-emergence damping-off of *Pinus resinosa* was directly correlated with increasing temperatures. Both types of losses were relatively negligible at an average temperature of 10° C., regardless of the amount of the inoculum used. This suggests the existence of a critical temperature below which *Pinus resinosa* may grow and escape infection by *Fusarium oxysporum,* an organism which otherwise causes relatively high losses.

Damping-off losses of *Pinus resinosa* and *Pinus sylvestris* were in direct relation to decreasing light-intensities, maximum final stands being obtained in full light. This condition was correlated with the increase in succulence of the hosts and in growth of three Fusarium lines used in the inoculations, with decreasing light-intensities.

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AN APPARATUS FOR THE GROWTH OF PLANTS UNDER CONTROLLED TEMPERATURE LEVELS

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INTRODUCTION

Many descriptions have been published of control chambers suitable for experiments on the relation of temperature to plant growth.[1, 2, 3] For the most part, however, the construction and maintenance costs of apparatus limit its general use to relatively few institutions. While such equipment has permitted plant studies under a wide range of conditions controlled by dependable instruments, some problems may be explored in equipment just as adequate for the particular purpose for which it is designed, at relatively much lower expense.

Most apparatus permitting temperature control has been constructed according to the incubator principle, providing single temperatures which are constantly maintained. However, this is in effect not comparable with natural conditions, where temperatures change according to seasonal conditions and fluctuate daily within seasonal levels through the influence of solar radiation. In order to study the temperature relations of plant disease, for example, and to plan experiments comparable to field conditions and upon which predictions and recommendations for nursery practice may be based, temperatures controlled at various levels should be permitted to fluctuate in a similar manner.

In the course of an investigation of the temperature relations of the Fusarium damping-off disease of coniferous seedlings, the writer[4] constructed and used an apparatus which successfully met these requirements and also embodied certain elements of design which made it adaptable to diverse problems in plant physiology, plant pathology, agronomy, etc.

DESCRIPTION OF APPARATUS

The apparatus, in its basic form, is a rectangular, insulated chamber, divided into compartments and bearing a heating unit at one end and a refrigeration unit at the other. Air circulates freely among the compartments, and in operation an air-temperature gradient is thus established over the length of the chamber, ranging from warmest in the compartment adjacent to the heater to coldest at the refrigerator end. Upon exposure of the interiors of the compartments to normal daylight, through glass tops, the

[1] Crocker, W. Organization, equipment, dedication. Contr. Boyce Thompson Inst. **1**: 9–52. 1925.

[2] Davis, H. R., and D. R. Hoagland. An apparatus for the growth of plants in a controlled environment. Plant Physiol. **3**: 277–292. 1928.

[3] Johnson, J. Constant temperature and humidity chambers. Phytopath. **18**: 227–238. 1928.

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temperature in each chamber then fluctuates about a different level, the greatest deviation from each mean temperature taking place during periods of maximum insolation and the least during periods of darkness.

Details of the construction are in figure 1. The chamber is built of three-fourths-inch white pine lumber, the bottom and four sides being double-walled, with a dead air-space packed with sawdust between. Half-inch plywood panels, inserted into grooves on the inner sides of the chamber, divide it into five compartments, which are covered by panes of glass with adjoining edges made smooth for close-fitting. The edges of the apparatus upon which the covers rest are further insulated with strips of felt. Additional compartments at both ends contain the heating and cooling units. These

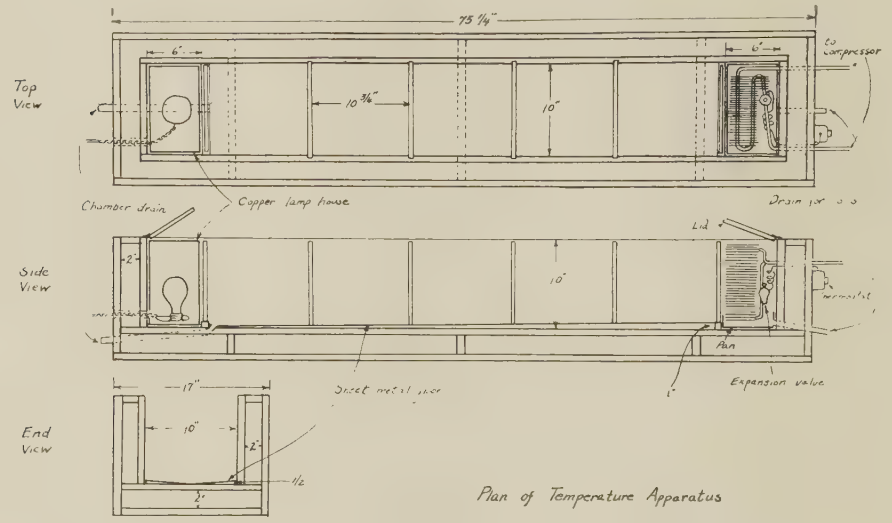

FIG. 1. Working drawings of elevations of the control apparatus.

spaces are separated from the interior of the chamber by additional panels and are covered by wooden doors inserted on the inner edges of the apparatus by hinges (Fig. 2, C, E). All panels are shortened at the top by one-quarter inch to permit free passage of air between adjacent compartments. In addition, the panels separating the spaces containing the heater and refrigerator units are also similarly shortened on the sides. These baffles thus permit free conduction of cold or warm air into the apparatus, at the same time preventing excessive radiation of heat into the chamber adjacent to the heater and freezing of solutions subsequently placed in close proximity to the refrigerator coils. Some panels bear holes permitting additional circulation of air (Fig. 2, B), which tends to space more evenly the temperature increments in the intervening compartments between the two extremes established at the ends.

Galvanized iron on the floor of the apparatus provides a graded surface in two directions (Fig. 1): a one-inch grade over the entire length and a half-inch slope from the edges to the center. Water drains towards an out-

let at one end of the chamber (Fig. 2, C, D). A drip pan and drain are also provided for the compartment containing the refrigeration coils (Fig. 1). Open seams within the chamber are caulked with putty to prevent the entrance of water into the sawdust-filled air space. All interior surfaces are painted with white enamel, for reflection of light within the compartments.

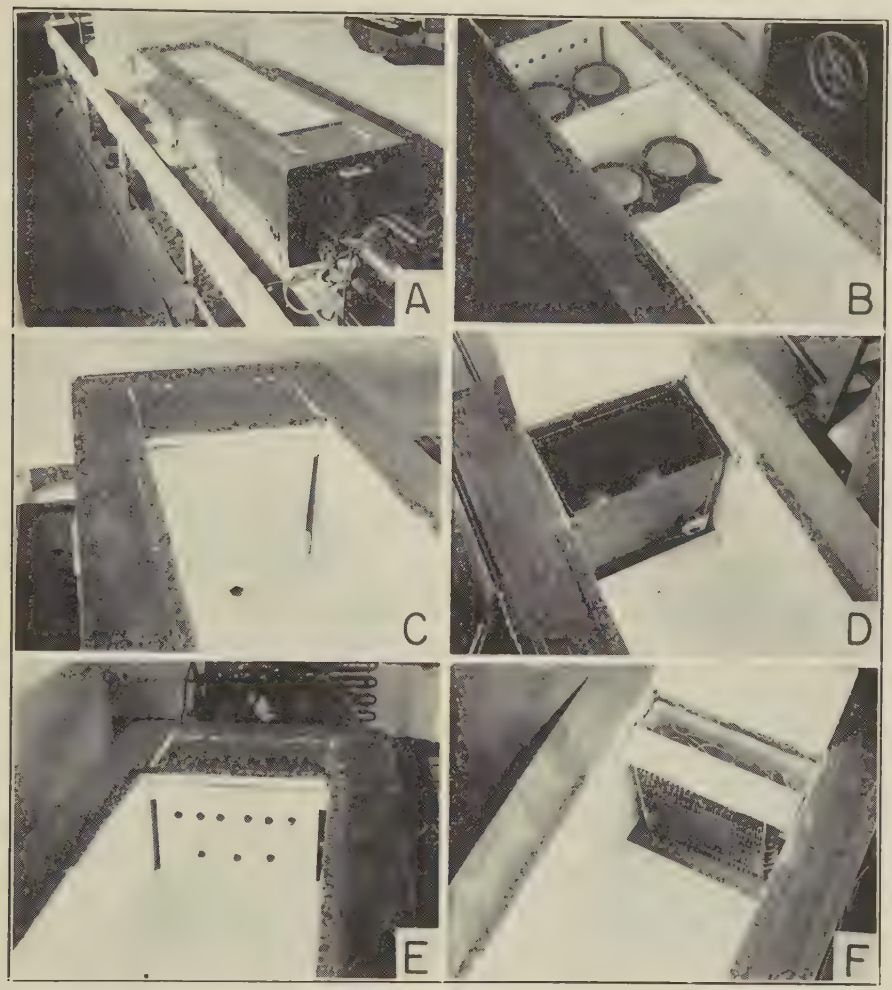

FIG. 2. A. View of entire apparatus. B. Enlarged view of equipment showing manner of disposition of pots of plants in the chambers. The glass cover of the central chamber has been removed. In the lower chamber, the manner of supporting pots on the sloping floor is illustrated. C. Warm end of chamber with lid and baffle in place. D. Same view as C with lid raised and cover of lamp house removed. The aperture for chamber-drain may be seen. E. Cold end of chamber, lid and baffle in place. F. Same view as E with refrigerator coil exposed.

Heat is supplied by electric bulbs housed in a copper container (Fig. 2, D), and the degree is controlled by varying the wattage of the bulbs. For the continuous and uniform supply of heat, the bulbs remain lighted for the duration of an experiment. Since ordinarily they are not visible, it is con-

venient to install in the exterior heater circuit an additional bulb in series (Fig. 2, A). A failure of this bulb to light indicates that the heating bulbs are inoperative.

Cold air is supplied by a small refrigeration machine equipped with thermostatic control, taken from a home-model refrigerator. The normal freezing coil is replaced by a double-pass fin-coil (Fig. 2, F) with an expansion valve control. The coil maintains low temperatures in the adjacent compartment (approximately 8° C. if desired) against the warm air coming from the other end of the apparatus.

The limits of the temperature range that may be established are thus conditioned by the relative degrees of heat and cold which are applied at the ends, and the spacing of the temperatures between these extremes in the intervening compartments is established experimentally by regulating the area through which air may circulate between adjacent chambers.

TEMPERATURE RECORDING

The actual temperatures in each of the chambers, in a 7-day test run with heat from a 20-watt bulb and a specific cold-setting, are shown in figure 3.

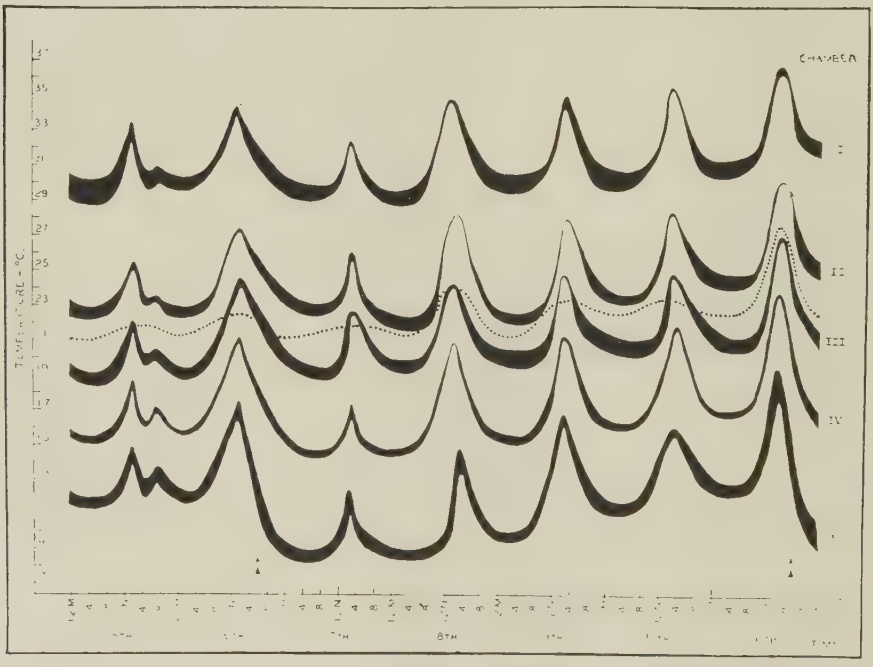

FIG. 3. Temperature record illustrating the levels of temperature secured within the air-control chambers over a period of one week, and the manner in which variation under the influence of insolation took place. Arrows indicate times of defrosting of refrigerator coils. The dotted line records the mean daily temperatures of the greenhouse during this period.

The width of the curve lines indicates the variance between the readings of 2 thermometers, suspended with bulbs at opposite ends of each compartment. Observations were hourly during daylight, and values for hours of

darkness usually were interpolated. In several 24-hour watches, after temperatures reached equilibria in darkness, they remained practically constant until daylight.

Diurnal fluctuations of temperature took place at different levels in the various chambers. There was a slight lack of uniformity for the two coldest chambers, whose values became generally higher as ice was deposited upon the freezing coil. Little change, however, was evident for 48 hours following defrosting (indicated in Fig. 3 by arrows). Consequently, in practice, the coils were defrosted every other day.

The sensitivity to variations in light intensity is evident. Maximum rise in temperature in the various chambers occurred during greatest insolation, bright days showing higher peaks than cloudy days. The sensitivity to light is evident in the record for the 5th day (Fig. 3). The morning and early afternoon were cloudy and dull, causing relatively slight rises in the temperature levels. In the late afternoon, however, the sun appeared very brightly, and as a consequence a secondary temperature rise in all the chambers was evident.

The levels of temperature attained in the several chambers of the apparatus and their fluctuations are independent of the external greenhouse temperature, which was controlled during the test.

The mean temperatures maintained in each chamber for the two days following defrosting—6th day, 6 P.M. to 8th day, 6 P.M.—and their standard deviations were: I, 31.5° ± 1.3° C.; II, 24.6° ± 1.6° C.; III, 20.9° ± 1.4° C.; IV, 16.4° ± 1.5° C.; and V, 10.4° ± 1.6° C. The standard error of any mean was 0.3° C.

COST AND ADAPTABILITY

The cost of the apparatus was comparatively low. Materials used in construction totaled approximately $25.00, and the price of the fin-coil, expansion valve, thermostat, and other installation expenses totaled less than $45.00. The compressor is relatively inexpensive, or else refrigeration may be furnished by other machines which often are standard equipment for many laboratories. Daily maintenance costs are almost negligible.

The equipment, as described, has been adequate for testing the relation of temperature to plant growth and disease,[5] when the particular purpose was to maintain a temperature range, each level of which duplicated natural conditions by increasing within its range under insolation. For other purposes, extensive modifications are possible. Constant temperatures may be secured, either by the exclusion of light, or, if light is essential, by the construction of a cover containing sources of light which produce no appreciable heat, as for instance, fluorescent filament-bulbs. In addition, a rheostat and thermostat included in the heating circuit would provide a more easily controllable source of heat.

[5] See footnote 4.

SUMMARY

An apparatus is described which permits the investigation of plant development simultaneously at several levels of temperature. The design of the equipment allows temperatures in separate compartments to fluctuate within their respective levels in a manner resembling the normal diurnal range of temperature in the field under the influence of varying degrees of insolation.

Details for the construction of the equipment are given. The cost of the materials is relatively low and subsequent maintenance expenditures are negligible.

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